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In defence of the anonymous referee

SINCE Professor Fraenkel-Conrat's plea for an end to anonymity in the evaluation of scientific manuscripts three months ago we have received several more letters on the subject. One or two have been published, but the majority have said little more than 'me-too' in tones ranging from the reasonable to the outraged and lengths ranging from one paragraph to ten pages. The question of anonymity in refereeing appears regularly in the correspondence columns of scientific journals, usually associated pejoratively with a cloak, and it is desirable that scientists give more than a passing thought to it every few years. In most other fields the arbitrator is not anonymous—justice, sport, criticism of the arts, industrial relations all maintain highly visible decision-makers. Can science alone remain aloof from requiring personal accountability in its judgements?

Those who advocate the removal of the cloak point to two benefits which, it is claimed, would immediately accrue. Referees would have to take their job more seriously since their judgement would be semi-public, and the stab-in-the-back opportunity for the malevolent referee would disappear. There cannot be an editor who wouldn't be glad to eliminate careless or mischievous refereeing, although its incidence may well be greatly exaggerated. On the other hand the debit side of removing anonymity is rarely mentioned.

One of the commonest misconceptions about refereeing is that it is done by a small elevated community. A careful study by Zuckerman and Merton (*Minerva*, 9, 66–100; 1971) showed very clearly that this was not true for *The Physical Review*, and although we have not the same impressive statistics to parade, the file of referees for *Nature* in 1973 contained more than a thousand names. Could we call on such numbers if the identities of referees were revealed? Only, we maintain, when the job was going to be the agreeable one of recommending the acceptance of excellent manuscripts. Large numbers of neither careless nor mischievous referees would send back, unreviewed, manuscripts which they believed to be inadequate and with those authors they had no desire to engage in combat.

Three points need emphasising.

First, referees should not be asked to endure face-to-face or even telephone encounters with irate authors. The sort of verbal roughing-up that even *Nature's* office staff occasionally have willed on them makes it clear that referees should not be placed in a position such that they can be got at by authors. The presentation of science is and must remain ultimately a written activity. This is not to deny the obvious power of the spoken word in education, exposition and elucidation. It is to deny the use of verbal battles, in which forcefulness, slick

presentation and quick wittedness are prized assets in resolving scientific disputes. It would be unfair to ask a referee to engage in conflicts on our behalf, and in any case most would quite reasonably decline to do so. This would result in the decision-making process falling back on a smaller core of conflict-hardened reviewers.

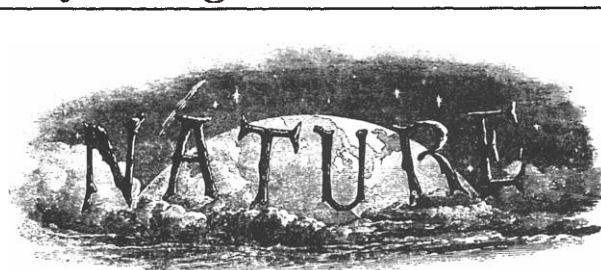
Second, not all the unpleasant things that are said from behind the cloak of anonymity are said from a desire to do mischief. The rather common home truth 'this is the tenth paper based on the same data that the author has attempted to get into the literature in the last year' may be motivated less from malice than a genuine dislike of the practice of multiple publication. Such things would be less easy to say without anonymity—some forms of truthfulness would be positively impaired by identification.

Third, the anonymous opinion, to the conscientious author, cannot easily be discounted. The referee serves more than as just the expert vouching for technical plausibility. He is also the potential reader vouching for relevance, wide appeal and readability. He is the one sample of 'readership' opinion that an author will normally go on. The value of these functions can be diminished by knowing the referee's identity and therefore dismissing his critique on purely personal grounds.

Would identification even do what its advocates claim: bring more conscientious and honest reviewing and deter mischief-making? The latter, probably yes, to a certain extent, although most of those already involved in mutual back-stabbing are well enough known to each other not to worry much about anonymity. Some of these combatants are also well enough known to us not to be allowed too frequent a battle. The former, probably no. The tendency would be just as much towards pulling punches and being less critical.

The tradition of depersonalisation in the assessment of manuscripts has, we believe, served scientific communication well. There are obviously flaws in the system, but radical steps to remove these flaws might remove instead a lot which is good.

100 years ago



THE NEW PHYSICAL LABORATORY OF
THE UNIVERSITY OF CAMBRIDGE

ON the 16th inst., at a congregation held in the Senate House, Cambridge, the Cavendish Laboratory was formally presented to the University by the Chancellor. The genius for research possessed by Prof. Clerk Maxwell and the fact that it is open to all students of the University of Cambridge for researches will, if we mistake not, make this before long a building very noteworthy in English science.

From *Nature*, 10, 139, June 25, 1874.



The Cavendish tradition

Professor Brian Pippard, present Director of the Cavendish Laboratory looks at its rôle in the forefront of physical research during the century of its existence.

ON June 16, 1874, the doors of the Cavendish Laboratory were formally opened. A hundred years later, the last members of the Department of Physics left in those buildings are preparing to move to new quarters, still to be called the Cavendish Laboratory, on Madingley Road.

Cambridge was not the first university in the British Isles to encourage experimental physics, and if in its earliest years the Cavendish stood out from the rest, it was more because of its first two professors, Maxwell and Rayleigh, than for any notable difference in outlook. Only after this, with the inspired appointment of J. J. Thomson at the age of 27 (not without some grumbling about mere boys being made professors), did the work begin to develop a special character. Of course the change was not sudden, but it is clearly revealed by comparing the publications in Rayleigh's day (before 1885) with those of 15 years later. Rayleigh at Cambridge is a different man from the gentleman scientist of Terling; he maintains the academic tradition of what we may call Natural Philosophy, undertaking with his students careful measurements to illustrate and guide mathematical reasoning, and to provide values of the constants of nature that pure reason alone cannot determine. By 1898 one has to search hard to find any trace of the earlier attitude; all is qualitative, the laborious search for patterns in phenomena beyond the reach of mathematical prediction. The names are revealing — Thomson, Rutherford, C. T. R. Wilson, Towns- end, Zeleny—the roll-call of early investigators of electrical conduction in gases. Thomson had started to work in this field as soon as he was appointed, had discovered the electron in 1897, and had built up a small team ready for action when the discovery of X rays and radioactivity swept away the old world of physics and inaugurated the golden age of string and sealing-wax.

Once more, let us remember that it was no sudden change; indeed, so far as the Cavendish was concerned these new effects principally gave new

strength to the attack on gaseous conduction. But Rutherford in McGill was developing that technique which is now looked on as the epitome of the Cavendish tradition. How much was his own natural gift, and how much he picked up of what was congenial in J.J.'s approach, is hard to resolve; at all events, by the time he returned to the Cavendish in 1917, with his finest achievements behind him, he found there an outlook compatible with his own, and proceeded within the next few years to develop the laboratory to a world-commanding eminence.

This is the accepted version of the myth, though one wonders if it is seen in the same light in Manchester, where Schuster was succeeded by Rutherford, and he in turn by Bragg and then by Blackett. It was in Manchester that the atom was born and first disintegrated, yet to the world at large it is the Cavendish that stands for British physics in that period. This is not the only example of the compelling hold that Cambridge exerts on the emotions of its alumni, and it is a self-perpetuating process whereby in due course the old boys send their best students back to seek their fortunes. The reputation of the Cavendish rests partly on what its professors have done, but more securely on what its students and junior staff are doing. So many of the country's most talented students are attracted to Cambridge by its reputation, to be joined, those who stay for research, by so many equally promising graduates from elsewhere, that the head of department's task is to encourage and to criticise, not to dominate. The conventional picture of Rutherford as something of a one-man band is quite misleading. It ignores the contributions of Chadwick and many others too numerous to mention, who proceeded to chairs and spread the Cavendish gospel of nuclear physics to the wide world; it also ignores the presence of Appleton, Bernal, Kapitza and G. I. Taylor, some of those who benefited from Rutherford's support without being drawn into his orbit.

Nevertheless, in comparison with the laboratory we now know, Rutherford's was an archaic monolith that could not survive the expansion of physics after the second world war, and Bragg soon recognised that the time had passed when one man could oversee the whole research effort. Inevitably he was conscious of stepping into a giant's shoes,

for it was many years before the Cavendish could free itself of the private feeling, and the world's expectation, that nuclear physics was the field it ought to cultivate above all others. But Bragg seized on the essential point that ideas are not the privilege of the leader but spring from the community of workers, and his decision to share responsibility among a number of virtually autonomous groups led to such successes as ultimately laid the ghost of the past. It was the seeds planted in the thirties by Kapitza, Bernal, Appleton, and Ratcliffe that grew in those years, so that by the end of Bragg's tenure Low Temperature Physics, Crystallography (especially what was to become Molecular Biology) and Radioastronomy were vying with Nuclear and Particle Physics to attract the best students. Devolution was complete; the laboratory was three times larger than at Rutherford's death, and its ability to contribute at the frontiers of research was now extended over a much broader field.

At the same time a certain weakness was becoming apparent. The experimental approach which we have traced to J. J. Thomson had developed into something of an antimathematical attitude; the followers of the prewar physicists, who had been amateurs in the best sense, were in danger of becoming merely amateurish in an increasingly professional world. And not only through disregard of essential theoretical techniques, but equally through nostalgia for the string and sealing-wax days when experiments were cheap and quick and, above all, for individual efforts. Thus, though some might have found a paradox in Mott's election to an experimental chair, and others welcomed it as a return to the ideals of Maxwell and Rayleigh, it is better seen as the recognition of the need to abandon parochial attitudes, such as the hope that physics will remain accessible to intuition and robust commonsense, with mathematics as an optional extra. Equally it was essential to realise that modern problems demanded modern equipment. Simplicity is for the man of genius; most of us have to do it the hard way. However much it may have wounded the pride of those who saw the Cavendish as something apart, it could only have been harmful to pretend that any single laboratory can go it alone nowadays. There will be local

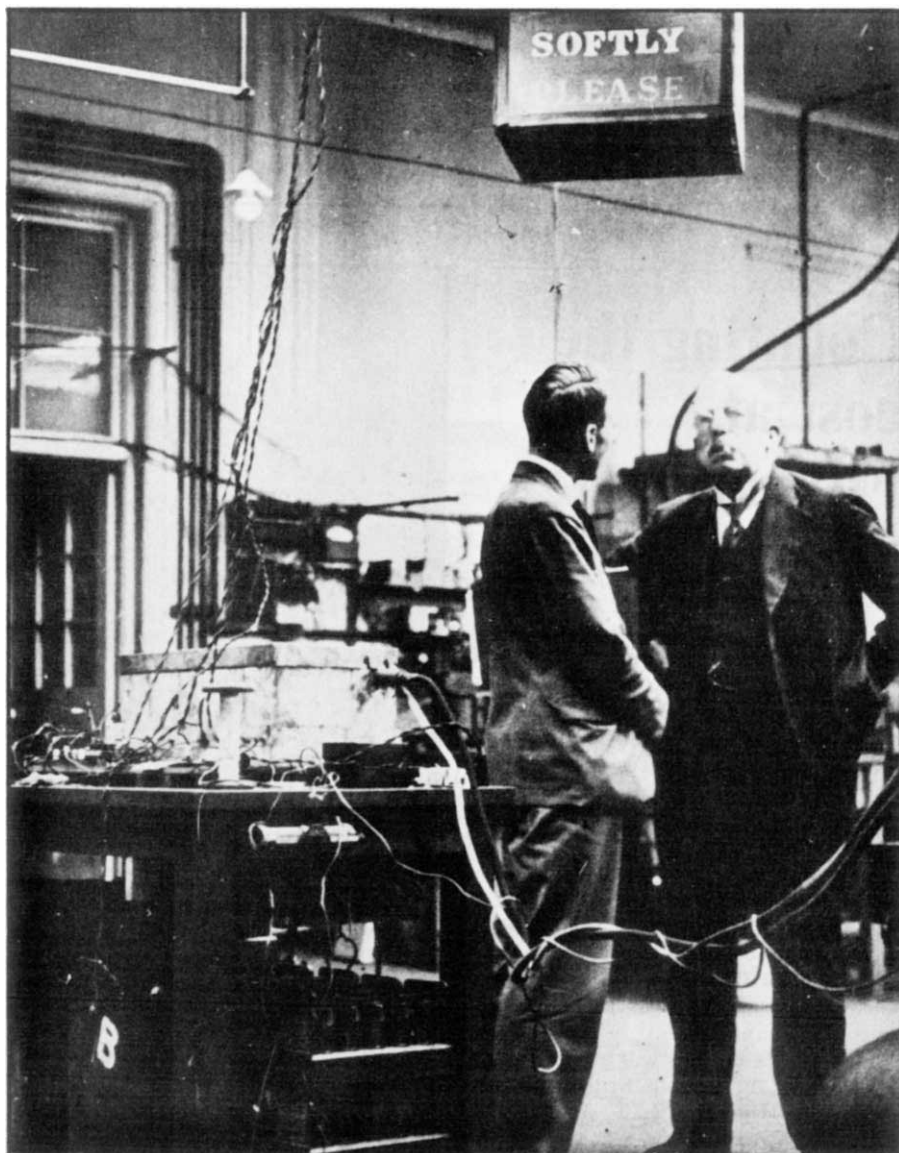
*Lord Rutherford talking softly to
J. A. Ratcliffe in the 1930s.*

traditions and national tendencies, but only as minor variants of an international style with, for example, British physics still leaning towards the experimental approach in contrast to the more mathematical tendency of the United States and Japan.

In keeping with this general trend many of the best contributions of the Cavendish in the last 20 years have been founded on technical innovations. The invention and exploitation of aperture synthesis for radiotelescopes, and the design and marketing of Sweepnik, the automatic scanner, are two very different examples of recent work in the old Cavendish style. Nor are there lacking descendants of the pre-war amateurs, who eschew the beaten track and find more scope in opening up new lines, where observation and measurement get little guidance from established formalism. If we ask which type of work seems to be more highly regarded, the answer is rather disconcerting. For we smile at Rutherford's division of science into nuclear physics and stamp collecting, not recognising all round us the modern counterpart in the esteem we accord to mathematical analysis and, at best, the grudging respect for the accurate description of phenomena.

In looking to the future we could do worse than ponder for a moment the danger that always threatens physics of degenerating into scholasticism, of valuing the formal structure above all, especially if it can be disguised as ultimate truth. No one, of course, will dispute the mastery of those who have contributed to our deeper knowledge of the fundamental laws, and there is no physics laboratory but is richer for the presence of a leader in these studies. It is another matter, however, for students to be seen switching off their attention when the lecturer embarks on a discussion of observations that do not fit into any tidy pattern; and we are all too familiar with the way in which, given the choice, the brightest students choose theoretical physics, leaving the more experimental options for the less gifted. This is the legacy of a generation of professional research workers whose collective learning has established a standard which demands of all recruits such detailed formal knowledge as will leave them no time and, worse, no desire to tread paths where the signposts are few and uncertain.

This is one of the challenges which



a laboratory like the Cavendish should attempt to meet. It has enough of a reputation to justify minor eccentricities of philosophy, and because of this, as well as the quality of its students, perhaps it has a greater responsibility than most to transit its version of the real nature of the subject. Its reputation never rested on knowing what everyone else had done, but on what its own members did, and how they inspired their students with the excitement of being a physicist. To carry on doing good research should not be too much of a problem, given that it is essentially a matter of catching and keeping as many as one can of the most ardent spirits, and providing the opportunity and encouragement for them to show what they are made of. But to carry this inspiration to the undergraduate level, where the need is greatest, is much harder than research—the urge is less, the praise fainter—and it is here that effort is really needed. To take a detached view, it could not be said to matter very much if most of the outstanding problems of physics were to

remain unsolved. Yet it matters that we should continue to try to solve them, and discover new realms to explore, if only to renew in ourselves, and pass on to others, the assurance that physics is much more than a collection of laws whose application is a matter of knowledge and routine. For physics is, besides, a living craft of hand and mind which is transmitted more by example than by rote. Embodying as it does a technique essential to fight the material problems of the world, it must continue to be taught, and taught as a craft. There are countries whose universities teach that science is what the textbooks say, and reduce their students to intellectual sterility; and there are not lacking in this country influential men who would promote the same disaster here, believing innocently that academic research is an expensive luxury. The challenge of our times is not to assert, but to prove, that this is a dangerous illusion. If the Cavendish has a role to play, it is once more its traditional role—to be in the forefront of the battle.

international news

Counting the cost at Flixborough

by John Wilson

TWENTY-EIGHT people killed, more than one hundred injured and upwards of £70m damage done to plant and surrounding property are the results of the most devastating explosion in peacetime Britain. It happened on May 4, at the 60-acre Nypro chemical factory in the village of Flixborough, Lincolnshire, which produces caprolactam—used in the manufacture of Nylon 6—and was caused by the sudden escape of a large quantity of cyclohexane vapour. The plant, owned jointly by Dutch State Mines (DSM) and the National Coal Board (NCB) was the sole producer of caprolactam in the United Kingdom. Its closure will affect 30,000–35,000 jobs throughout British industry to varying degrees.

A most disturbing aspect of the disaster is the damage outside the factory gates—thirty families have had to be rehoused—and questions are being asked in parliament and elsewhere about the safety of other large chemical installations in Britain, and indeed throughout the world (Nypro owns similar plants in Holland and Augusta, Georgia). The Secretary of State for Employment, Mr Michael Foot, has ordered a public inquiry into the disaster and the British Chemical Industry Safety Council are to review its findings. Meanwhile, Mr Jack Jones of the Transport and General Workers Union has said that the union will hold a separate inquiry of its own. Twenty-one of the dead were members of the union and Mr Jones believes that the survivors will talk more freely to their own union-appointed investigator.

But more than a week after the disaster, explosions were still taking place on the site and firemen had not penetrated through the pools of dangerous chemical to the control room where many vital records were kept. It will probably be a long and difficult task to reconstruct exactly what happened.

What is now almost certain, however



is that the escape of cyclohexane vapour occurred at that part of the plant concerned with the oxidation of cyclohexane to cyclohexanone oxime, an intermediate product. High temperatures and pressures are involved in this process. One of the units was under repair at the time and a bridging pipe is thought to have fractured, although how the gas ignited is not yet known. But Mr Leslie Grainger, NCB board member for science, says that the way the gas escaped is more important than its ignition. In spite of searches for cigarettes and matches, the banning of motor vehicles and the issue of special anti-static clothing, most experts are agreed that the risk of a random spark cannot be entirely eliminated. The effects of any explosion, however, can be minimised by the design of the plant. The established practice of piping volatile material in the open in the hope that

Flixborough: no rebuilding?

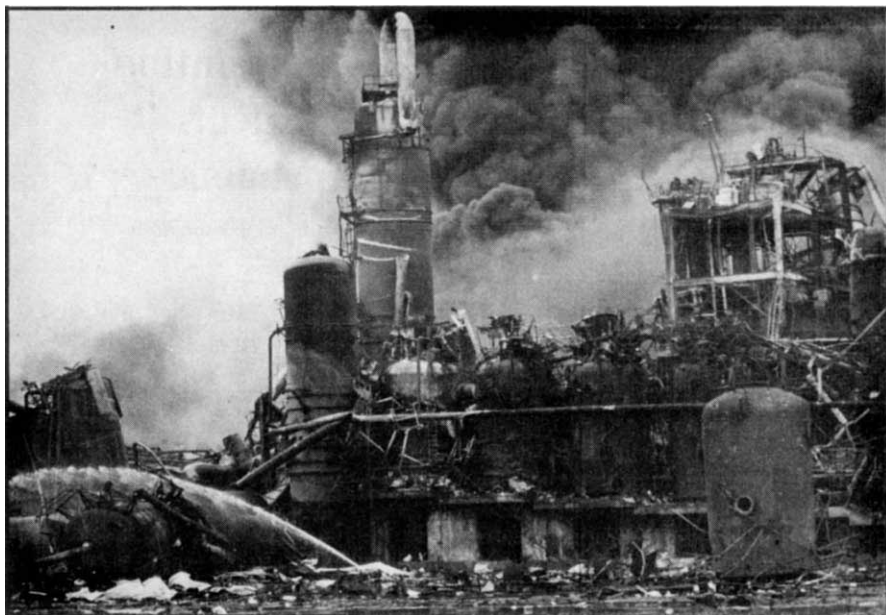
any leak will be dispersed, must now be revised.

The greater the degree of physical connection between various processes at risk, the greater the danger that more than one unit will become involved in an accident. Even the siting of other processes, the manufacture for hydrogen, for example, may be vital in an emergency. The Nypro control room and laboratories were built in the centre of the site, between the oxanone processes and the hydrogen plant. Obviously some degree of connection is necessary—that is what a petrochemical complex is all about—but there must come a point when safety factors outweigh engineering expediency. The difficulty, says Mr Grainger, is to maintain the economic links without the physical ones

The NCB became interested in the Flixborough venture when it was seeking an outlet for its crude benzol (a source of cyclohexane) produced at coke ovens up and down the country. Its opportunity came when Fisons Ltd, the fertiliser manufacturer, withdrew from partnership with DSM in 1971. By December 1972 a new £15 million extension to the plant was completed, producing caprolactam from cyclohexane; up to that time phenol had been used as a raw material.

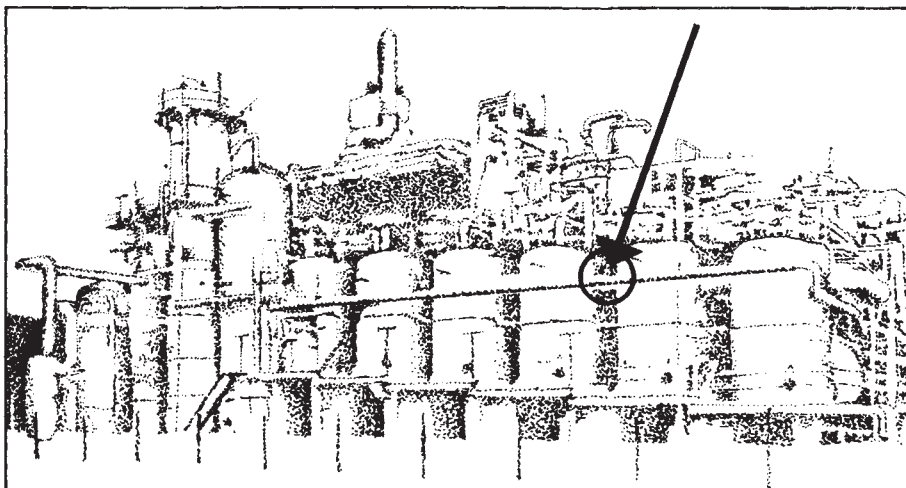
The cyclohexane was oxidised to cyclohexanone and the oxime formed by the addition of ammonia and sulphuric acid. Ammonium sulphate used to be a principal by-product of this reaction—which is why Fisons were involved—but was later recycled as phosphoric acid. The oxime was finally converted to caprolactam by Beckmann isomerisation.

A third phase of expansion was to have been commissioned later this year but whether that expansion would have used phenol or cyclohexane had not been decided at the time of the explosion. Mr Grainger says that there is now an emotional argument against the cyclohexane technique but hopes that any further decision will be based on rational grounds. Whatever process might be used in the future, there is strong local opposition to rebuilding the site at Flixborough. Comparisons are inevitably drawn in any industrial disaster of this size with the safety of nuclear installations. These have greater safety restrictions placed upon them and in Britain they need the approval of the Nuclear Inspector, whose decision is final. But a chemical plant may be built without consulting anyone except the local planning authority, who usually have little expert opinion to draw on. The Factory Inspectorate has no say in the matter until the plant is erected. At a time when the safety of nuclear power plants is in question, this seems to be a double standard.



THE oxanone plant at Flixborough, where the leak which caused the fatal blast is thought to have occurred. The picture above shows the cyclohexane containers after the explosion. One tank had been removed from its place for repair, and in the space it had formerly occupied a bridging pipe was

used to connect the remaining tanks (location arrowed in drawing below). Nypro Ltd refused to comment on the suggestion that temporary piping was the source of the leak. They are waiting for the report of the Factory Inspector which will be published later this year.



WHATEVER the precise cause of the Flixborough disaster may turn out to be, Sir Brian Flowers, Rector of Imperial College, London, and Chairman of the Royal Commission on Environmental Pollution has set wheels in motion within Imperial College to find out whether teaching there places sufficient emphasis on reducing technological hazards of all kinds to an absolute minimum. Sir Brian expressed concern in a speech in April that "what is regarded as an acceptable disaster is increasing all the time, whether it is a hijacking or a motorway pile-up . . . It seems strange that increasing acceptance of technological failures should seem to coincide with increasing in-

Teaching for safety in technology

by Roger Woodham

tolerance of environmental pollution." So the Flixborough disaster was the catalyst but not the cause of the setting up of a working party under Professor A. R. Ubbelohde, Head of the Department of Chemical Engineering and Chemical Technology at Imperial College.

Professor Ubbelohde said last week that the ultimate objective must be to imbue engineers—be they recent graduates or those with industrial experience

returning to Imperial College for post-experience courses—with the "concepts of safe design". He also predicted that the monitoring of potential hazards, preferably by two independent methods, will receive much more attention in the future.

Sir Brian says that all too often "clever economic solutions have come before the safe solution which respects life." Modern technology should be fail-safe (so that alterations in any parameter of the system make it safer) but it is not, he said.

The working party has three months to submit a report and it seems unlikely that the document will stay internal to Imperial College for very long.

EVER since Arab oil producers shut off oil supplies to the United States late last year, President Nixon has been telling the American public at every opportunity that his Administration is committed to a pell-mell drive to make the United States self-sufficient in energy. Called Project Independence, the programme was launched with a flood of rhetoric in November 1973, and President Nixon still maintains in his public announcements that the goal will be met by 1980. But last month, two independent studies concluded that the effort will be extremely costly, and that self-sufficiency will probably be unattainable—even by 1985.

With much of the press distracted by more topical matters, the studies drew little public attention but they provide the strongest evidence so far that the United States will have to rely on imported oil to meet its energy demands for at least another decade and probably for long after that, in spite of massive investment in research and development and capital equipment.

Prepared by the Massachusetts Institute of Technology's Energy Laboratory and the National Academy of Engineering (NAE), the studies analysed Project Independence from two different standpoints but both reached essentially the same conclusion. The MIT study argued that energy self-sufficiency can only be bought with another sharp increase in the price of domestic oil which, in the short term, "would yield little more than an income transfer from consumers to producers", while the NAE study concluded that it will take capital expenditure amounting to about \$600,000 million over the next 10 years and government intervention in the energy industry to make the United States independent of foreign energy supplies by 1985.

If anything, the NAE study paints the bleaker picture, for it simply examines what will be needed from each energy sector between now and 1985 to give Project Independence a chance of success, and it comes up with some pretty sobering conclusions. The burden of the study's message is perhaps best summed up by the following statement in the NAE report: "Achieving the complete range of programs described in this report by 1985 is not considered . . . to be of high probability. Even if it is accomplished, the United States would be buying time. For beyond 1985 there looms the ominous prospect of even greater demands for energy from ever-increasing and ever-rising expectations from home and abroad".

Although the Administration's statements about Project Independence have so far been unencumbered by a definition of what actually constitutes self-sufficiency, and President Nixon seems to pay little attention to advice from

Gloomy outlook for Project Independence

Colin Norman, Washington



his top energy officials that 1980 is a totally unrealistic date to expect the United States to become independent of foreign energy supplies, the effort required will be staggering.

For a start, energy supply and demand predictions made before the energy crisis began to bite indicated that by 1985, Americans would be consuming the equivalent of 58 million barrels of oil every day, while domestic supplies would amount to little more than 40 million barrels. Somehow, therefore, Project Independence will have to contend with a shortfall of about 18 million barrels of oil equivalent a day within the next 10 years.

According to the NAE study, this could be achieved by an aggressive conservation programme and a crash effort, beginning this year, to exploit all domestic energy resources in the United States. The conservation effort could be expected to reduce demand by about 8 million barrels a day, while the development efforts would entail doubling coal production, massive strip mining in the Western United States, increasing nuclear power output so that fission reactors generate a third of the total electricity output in the country (up from less than 1 per cent last year), increasing oil and gas production by 25%, and establishing entire new industries to produce synthetic petroleum and natural gas from coal and oil from shale.

Apart from the \$600,000 million price tag, there are several major obstacles which are likely to prevent Project In-

dependence from being a success, however its goals are defined. For one thing, such a massive development of energy resources is likely to be achieved at the expense of considerable destruction of the environment, and for another, the NAE study suggests that there is likely to be a considerable shortage of skilled manpower, materials and equipment. In short, Project Independence is likely to be an expensive failure when measured against President Nixon's public announcements.

As for each energy sector, the NAE study comes up with the following conclusions:

- **Coal.** The United States has about one third of the world's coal reserves, and these are likely to provide the bulk of any increase in domestic energy output in the next decade. According to the study, output must be doubled by 1985, chiefly by opening up large federally owned areas of Montana and Wyoming for strip mining and expanding underground mining in the Eastern United States. By itself, an increase from 660 to 1,260 million tons a year over the next decade doesn't sound too daunting a prospect, but when translated in terms of the physical facilities which will have to be added to the coal industry, the undertaking is staggering.

"Stated another way", the report says, "on the average one new deep mine and one new surface mine must be brought into production every month for 10 years. In contrast, only 13 mines of greater than 2-MTPY (million tons per year) production were opened in the 10 years from 1960 to 1969".

Equally startling are implications for transporting coal to power stations and other users if output is doubled. The NAE study suggests that 60 new rail-barge systems, between 100 and 150 miles long and each capable of handling 2 MTPY, will have to be constructed in the eastern United States, and 70 3-MTPY systems of between 1,000 and 1,200 miles will have to be constructed in the West. To haul the stuff, 8,000 locomotive units and 150,000 hopper cars, each of 100 tons capacity will have to be built. At present there is a severe shortage of hopper cars.

- **Oil and Gas.** Last year, more than 75% of energy requirements in the United States were supplied by oil and gas, but increases in domestic production will only come from very costly new sources such as offshore deposits in the Atlantic and in Alaska, and the NAE study predicts that at best an increase of about 25% can be expected from United States sources. To achieve even that increase would cost about \$180,000 million over the next decade: it would require an average of 58,000

new wells to be drilled each year—twice the rate in 1973—new methods for recovering oil from marginal wells would have to be developed and environmental problems associated with offshore production would have to be resolved. But even if all those programmes are successful, domestic oil and gas production would reach only about 27 million barrels per day equivalent in 1985, which is about 1.6 million barrels short of the demand in 1973.

● **Electricity production.** The total generating capacity of the United States electrical industry in 1973 was about 435 million kW and the NAE study concludes that this could be doubled over the next 10 years with capital investment of about \$300,000 million. First, nuclear power would have to be expanded greatly above present projections, to reach about one third of total electrical capacity by 1985. This will require between 2 and 3 new nuclear units to be built every month, uranium production would have to be expanded by a factor of 6, between 30,000 and 40,000 plant operators and maintenance personnel would have to be trained, 30 reactor pressure shells and turbine generators will have to be manufactured and delivered each year, and the time it takes to license and construct nuclear plants will have to be greatly reduced from the present 10-year average.

Another key programme in the electrical energy sector is the expansion of coal-fired power station capacity. This, the NAE study suggests, will require five oil-fired power stations to be re-converted to coal every month for the next four years, two new 700 MW stations will have to be brought on line each month for the next decade, and 10 new plants will have to be constructed to produce medium-BTU gas from coal to fuel 50 power stations which are now fired with natural gas. All of that, however, would have to rely on the development of new technologies to control the emission of sulphur dioxide from power plants, or alternatively, environmental regulations would have to be greatly relaxed.

● **Synthetic petroleum, natural gas and shale oil.** In order to meet the predicted shortfall in oil and gas, it is estimated that new technologies must be developed to produce the equivalent of 1.1 million barrels of oil per day from coal gasification, 0.6 million barrels per day of synthetic petroleum from coal, and 0.5 million barrels per day of shale oil. Apart from a massive research and development programme, it will take a capital investment of between \$16,000 and \$22,000 million for the coal conversion projects, and between \$3,000 and \$5,000 million for the shale oil production.

The NAE analysis points out that the cost of those processes is likely to deter early production, and constraints such as the availability of water and environmental regulations are likely to set a limit on the ultimate capacity of some of the processes. And the MIT study is even more pessimistic, for it states that at present oil prices, there is insufficient financial incentive for the energy industry to move into those fields. Both studies therefore recommend that the federal government should provide a variety of incentives such as guaranteed prices, tax breaks and joint financing of development plants to bring the technologies on line as early as possible.

● **Conservation.** The prospects for the success of Project Independence hang to a great extent on whether or not the United States' insatiable appetite for energy can be curbed. Although the rapidly increasing cost of energy will work to reduce demand through the normal market forces, it is clear that the federal government must take a strong lead in encouraging conservation by a variety of measures such as allocation of funds to mass-transit systems, promoting research and development on more efficient ways to produce and transport energy, and by continuing such measures as speed limits on highways and encouraging car pooling.

The NAE study identifies four chief constraints which lead to its prediction

that it is highly unlikely that all the projects necessary to achieve energy independence will be carried out. First, the capital investment required—\$600,000 million over 10 years—is almost double the present investment rate in the energy industry, and it should be compared with the total investment in all industrial plant and equipment of about \$100 million in 1970. Second, the availability of water is "a serious concern for producing oil from shale or synthetic fuels from coal". Third, environmental damage resulting from strip mining—particularly in the remote Western coalfields—and from burning large quantities of coal will have to be overcome by the development of new technologies, otherwise environmental regulations will have to be relaxed. And finally, a huge manpower problem could arise since several hundred thousand people will have to be recruited and trained in the energy industry during the next decade; in particular, the NAE study points out that enrolment has been dropping in university engineering courses.

One thing is clear from the study—a number of complex decisions must be taken by the federal government within the next year, and a coherent national energy policy must be formulated. So far, however, neither the Administration nor Congress has matched Presidential rhetoric with concrete action.

ESRO awards Spacelab contract

THE European Space Research Organisation has decided to award a contract worth approximately 180 million accounting units* for the design and development of the manned orbital laboratory Spacelab to VFW-Fokker/Erno of Germany, as prime contractor for a European industrial team whose composition reflects the financial contributions to the Spacelab programme by participating ESRO member countries. The unanimous decision was made by ESRO's administrative and finance committee, composed of member country delegates, at a meeting in Paris. It followed six weeks of evaluation by ESRO officials of two industrial proposals of high technical quality.

This six-year contract provides for the delivery of one Spacelab flight unit, fully qualified and ready for the installation of experiments, by April 1979. Other main deliverable items are two engineering models, three sets of ground support equipment and initial spares.

The Spacelab project is the most important cooperative programme between ESRO and NASA. ESRO is responsible for the design, develop-

ment and construction of the laboratory, which NASA will launch in its space shuttle and operate. The first Spacelab launch is scheduled for early in 1980. NASA will soon commit itself to purchase a second Spacelab unit from ESRO and has indicated its intention to purchase such additional Spacelabs as may be required.

Unlike earlier space laboratories, Spacelab will be reusable. It will be designed for 50 missions of a week to a month, or a nominal life of 10 years. It will enable, for the first time, scientists, engineers and technicians to travel and work in Earth's orbit without undergoing intensive astronaut training. Spacelab is designed to reduce significantly the cost and time needed for space research and its applications, and to provide to a large number and wide variety of American and European users a versatile laboratory in which experimenters can work in comfort.

* At mid-1973 price levels 180 MAU is equivalent to approximately £95 million, FF 1,000 million, DM 578 million and \$226 million.

correspondence

Earth scientists

SIR,—‘European earth scientists disunite’, an article by Peter J. Smith in your issue of April 12 is erroneous and misleading; we are concerned that such an article should have appeared in *Nature* without an attempt to check the facts.

The main tenor was implication of lack of coordination between the organisers of a forthcoming meeting in Reading of European Geological Societies (the Geological Society and the University of Reading) and the European Geophysical Society. In fact the minutes of the first meeting of the Policy Committee for the Meeting of European Geological Societies which is to take place from September 8-12, 1975, were sent in July 1973 to the Secretary of the European Geophysical Society at the Royal Society. A letter with his comments was received. It was certainly not “more or less by accident” that he discovered that there would be such a meeting. In December 1973 a special letter was sent to the President of the European Geophysical Society, with a copy to the Secretary, inviting participation of members of EGS and requesting their views and suggestions for topics for discussion. The letter was acknowledged by President Morelli welcoming the chance for cooperation, and expressing the hope that many members of the EGS would be interested in attending the Reading meeting.

For one whose professed anxiety is about the unity of earth science (that is, of geology) Mr Smith lays a surprising amount of emphasis on a distinction between geophysics and geology. We would expect him to recognise, as we have for many years, that geophysics is a necessary and important part of geology. Phrases such as “there being no such thing as the geology of the core” make semantic nonsense to stratigraphers, mineralogists and sedimentologists like us. Of course we agree that geophysicists, tectonicians, geochemists, palaeontologists, petrologists and the rest need to talk to one another: that is the object of the projected Reading meeting in 1975; we do not, however, see it as a matter for geophysicists on the one hand and “geologists” on the other. The theme chosen for the 1975 meeting, ‘Europe from Crust to Core’ is designed to secure the widest possible interdisciplinary discussion. As to the

formation of a Pan-European Society of Earth Scientists, that is not the immediate object of the exercise, but if some of those attending wish to discuss such a possibility, so well and good.

PETER KENT

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PERCIVAL ALLEN

University of Reading

Reply from Chile

SIR,—In your journal (March 29) you published a letter sent to you by well known scientists who referred to the “dramatic conditions of the Chilean academic community”. As these scientists have great influence in the scientific community, I must reply to such a letter.

I was in fact nominated as Rector of Universidad Católica de Chile by the Junta Militar de Gobierno, which designation was confirmed by a Decree of the head of the Catholic Church in Chile and Gran Canciller of this University, Cardinal Raul Silva Henríquez. Nothing said in the letter referred to, corresponds to the real situation in this University since the day I assumed office, early in October, 1973.

Contracts of academics are not provisional as the letter suggests. It is true that some of them have been dismissed although no special Commission has existed for this purpose. They were dismissed because they simply abandoned their job or because they never anticipated in the academic life of this University or any other but preferred political activities instead of doing the work they were paid for.

In any case, the number of academics dismissed from this University is less than the figure of 65 quoted in the letter. This is out of a total of over 2,000 teachers. In all cases of dismissal full legal compensation has been paid. None are natural scientists; they all belong to the social sciences, mainly sociology and political science.

None of the 11,500 students in this community has been dismissed and so there has been no need of reregistration.

With regard to the staff in prison or executed, some teachers and administrators have indeed been questioned. Very few are still in prison (only 5) and legal procedures are followed to clarify their responsibilities for the crimes of which

they are accused, according to traditional Chilean law. They have been allowed legal defence, as it is a long standing custom in Chile which nobody here has forgotten. As to the executions which they claim have happened, I would like to hear of one member of this University who has suffered, for I know of none.

As to the departments which have been dismantled, none of the examples given in the letter has occurred here. No Faculty or Institute has been closed except for two political centres whose Marxist political inspiration was dedicated to public indoctrination.

Nevertheless, I can say that those who judge us seem to ignore the facts and to forget our previous history. They have not considered at all what has really happened in the University. They have not considered that the Supreme Court and the Congress had previously declared the former government illegal, nor that our Armed Forces have historically been politically neutral, nor the people's claim over the last two years to remove the Marxist regime which was destroying all our rights and most beloved traditions. Scientists particularly, should be aware that proof is required before any decision. Nobody should say anything which he may regret the next day; nobody should allow themselves to be the instrument of others hidden intentions.

In this sense, I trust that time and history will establish what really happened in this country, despite all intentional and international campaigns against Chile.

JORGE SWETT MADGE

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Penicillin

SIR,—Your article (*Nature*, May 24) on cancer research by Brian Ford contains the statement that it was the war effort and directed research which gave us penicillin. This statement is incorrect. The reinvestigation of penicillin which led to the discovery of its curative properties was started in 1938, well before the outbreak of the war, and its aim was not the practical one of discovering a new chemotherapeutic agent.

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news and views

Rabbit—scallop transplants

COMPARING and contrasting animals of widely different form is a common pastime for muscle specialists, since in their field similarities generally outweigh differences and there is much common ground for detailed correlation. In general, muscle contraction takes place by some kind of sliding filament mechanism, that is, by relative movement of actin and myosin filaments, and regulation of the system is mediated by the level of calcium ions. Within this fundamental framework, however, there is considerable variation of detail in the Animal Kingdom—in the way, for example, the calcium control is exercised. In vertebrate skeletal muscle, changes of calcium level affect the muscle activity by modifying the troponin and tropomyosin components of the actin filaments, whereas in the muscles of molluscs and some primitive invertebrates myosin and not actin is controlled. In some invertebrates there is dual control, that is, both types of filament are involved in regulation. Evolutionary forces are clearly at work here, and the understanding of problems of animal specialisation and adaptation provides an interesting background to the more fundamental task of elucidating the molecular basis of muscle contraction.

Myosin is a protein with a long tail portion and two globular heads. From the heads may be dissociated polypeptides of relatively low molecular weight, the so-called light chains. In rabbit skeletal muscle myosin there are two classes of light chains, one class released by alkali and the other by DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)). The alkali light chains have been shown to be involved in the enzymatic activity of the myosin, but the function of the DTNB light chain has long remained a mystery. Scallop muscle myosin, on the other hand, has light chains released by EDTA, and without these chains the splitting of ATP by the myosin-actin system is not susceptible to changes of calcium, that is, the myosin becomes 'desensitised'. Is it possible, therefore, that the DTNB light chain in the rabbit has a role analogous to that of the EDTA chain in the scallop? If it does play a part in calcium regulation, how significant is its function relative to the actin-linked control? Perhaps the existence of the DTNB light chain is vestigial, an evolutionary relic of the more primitive scallop type of myosin regulation that has been usurped by sophisticated actin control? To test these ideas, Kendrick-Jones (see page 631 of this issue of *Nature*) has carried out molecular transplants in which the DTNB light chain from rabbit is grafted onto desensitised scallop myosin. Sure enough, the sensitivity is retrieved. This is a most remarkable result, and a triumph for advanced protein techniques which, together with the investigator's skill, can bridge a gap that was many millions of years in the making.

The DTNB light chain restores the calcium sensitivity more fully to the intact scallop myofibrils that have been treated with EDTA than to preparation of pure scallop myosin similarly treated. There is no evidence that the DTNB reacts directly with any protein other than the myosin, however, and it is possible therefore that actin in the myofibrils may have an indirect effect by stabilising the myosin in some way. Experiments with radioactivity labelled DTNB light chains show that one mole is bound per myosin

molecule. Since the myosin molecule normally releases one mole of EDTA light chain, and this must be removed before the DTNB chain will attach, the binding sites for the two polypeptides are likely to be essentially the same. The binding of the DTNB light chain is tight and specific, and the other rabbit light chains—the ones released by alkali—cannot be made to bind to scallop myosin or to affect its calcium sensitivity.

Since rabbit turns on scallop, what can the latter do in return? Kendrick-Jones has not omitted this intriguing alternative, but, disappointingly, the full reciprocal relationship is not established. Although radioactive labelling shows that the scallop EDTA light chain will bind to rabbit myosin that has been treated with DTNB, the myosin does not then become calcium sensitive. Still, the fact that the two chain types are interchangeable is interesting enough, and the interest is intensified by the knowledge that their chemical structures have no obvious similarities.

There is little direct evidence for myosin playing a part in the regulation of vertebrate skeletal muscle activity by calcium. X-ray diffraction of stretched living muscle has indicated that the positions of the myosin cross-bridges alter when the calcium level is changed. When, however, the myosin is extracted from the muscle, its ability to split ATP in the presence of pure actin is unaffected by the calcium level, though it does bind small amounts of calcium. The fault may lie here in the extraction and purification procedure.

Some living organisms have myosin-linked regulation, some actin-linked, and some have both. The transition and gradation between these regulatory mechanisms, their evolutionary significance and what particular physiological advantages are conferred by the possession of one or other or both remain fascinating questions. It could be argued, however, that the scope at least of the problem has been encompassed by Kendrick-Jones's successful matching of the light chains separated by aeons, and union of two animals sufficiently dissimilar to startle even Edward Lear.

E. J. O'BRIEN

Aspects of the asthenosphere

It may be a cliché to say that the Earth's asthenosphere exerts a profound influence on the crust and lithosphere above, but it is neither less true nor less important a statement for that. Irrespective of whether convection currents are limited to it or go deeper, the asthenosphere clearly plays a crucial part in the horizontal motion of continents and ocean floors; it is the source of at least some of the material which finds its way from the mantle to the Earth's surface, and is closely related in composition to that part of the mantle which provides the rest; and it is the ultimate means by which a crust with a changed load may achieve isostatic equilibrium. In other words, it governs the behaviour of the upper layers of the Earth to such a degree that the determination of its physical and chemical properties can be safely said to be one of the genuinely fundamental problems in the Earth sciences.

And for obvious reasons it is a problem both difficult

and complex—characteristics which provide considerable opportunity for disagreement. On the other hand, there are some things that can be said with little fear of contradiction. Presumably few people would now object to, for example, the general definition of the asthenosphere as a layer with no enduring resistance to a shearing stress and thus with a finite strength small enough to be neglected (Le Pichon *et al.*, *Plate Tectonics*, Elsevier, 1973). It is interesting to note, however, that modern as this definition may sound, the possibility of flow inherent in it had actually been implied by the acceptance of the principle of isostasy more than a century ago; and it was the problem of isostasy which later led Barrell (*J. Geol.*, **22**, 441 and 655; 1914) to incorporate the flow property into a layer model involving a 'lithosphere' and an 'asthenosphere'. In other words, by a curious coincidence the concept of an asthenospheric layer came about independently at almost exactly the time that Wegener was proposing continental drift, the phenomenon that would later require the characteristics of the asthenosphere to provide a convincing mechanism.

But it was to be a long time before the asthenosphere would be properly defined in non-isostatic terms, even though the process began with Gutenberg's early suggestion (*Z. Geophys.*, **2**, 24; 1926) that the general monotonic increase in seismic velocity with depth in the Earth may not be applicable to the uppermost mantle. Much later, in the light of many more and more modern seismic investigations, Gutenberg (*Physics of the Earth's Interior*, Academic Press, 1959) was able to propose the existence of an upper mantle low velocity zone having a minimum P wave velocity at a depth of about 100 km and a minimum S wave velocity within the depth range 100–200 km, the maximum decrease in velocity being a few percent for each type of wave but slightly greater for S waves than P

waves. It now seems doubtful whether the P wave channel is universal, but the more pronounced S wave low velocity zone has been confirmed. It probably begins at a depth of 70–80 km beneath typical ocean basins and in the range 110–130 km beneath continental shields, in each case ending at a depth of about 250 km (Press, *J. geophys. Res.* **75**, 6575; 1970). The viscosity in the vicinity of this zone is also apparently low, both Gordon (*Geophys. J.*, **14**, 33; 1967) and McKenzie (*Geophys. J.*, **14**, 297; 1967) having estimated a value of about 10^{21} poise which thereafter rises very rapidly with depth. In summary, then, the asthenosphere seems to be a relatively thin upper mantle layer which varies somewhat in depth and thickness according to the tectonic environment above, in which seismic velocity is anomalously low (and seismic attenuation high), and in which viscosity is also particularly low.

But how does this peculiar combination of properties arise? According to Ringwood (in *The Earth's Crust and Upper Mantle*, American Geophysical Union, 1969), there are several ways in which zoning in the upper mantle might take place. One (mineralogical zoning) is based on the ability of material with his hypothetical 'pyrolite' (1 part basalt, 3 parts dunite) composition to crystallise into four distinct mineral assemblages with different physical properties. Which of these will form at any given time and place will be determined largely by the intersection of geotherms with the assemblages' stability fields; in other words, the assemblage forming will be the one stable at the relevant temperature and pressure. And since temperature and pressure vary with depth in the mantle, so will physical properties. A second possibility is chemical zoning in which "the extensive fractional melting and upward segregation of low-melting components that is inferred to have occurred beneath highly evolved con-

Recognition of *lac* initiator

PROTEIN synthesis in *Escherichia coli* begins after the ribosome and transfer RNA_i^{Met} have bound to a region of the messenger RNA containing the initiator triplet codon. An intriguing question is how the machinery recognises and selects the correct AUG (or GUG) codon out of others that may be fortuitously or otherwise present. Maybe a specific sequence of bases is recognised or maybe there is some aspect of the secondary structure with a recognisable shape.

An approach to this problem is to make use of the fact that initiation complex is stable in the absence of other charged tRNA species so that protein synthesis is prevented from taking place. It has been found that a region of the messenger RNA is protected from attack by endonucleases by the bound ribosome and it is tempting to believe that these protected regions contain the features that are recognised as well as the initiator codon.

The base sequence of several such ribosomal binding sites have already been published for various cistrons of various phages and a possible initiator triplet has invariably been found. In some of these sequences the protected sequence looks as if it could form a region of helical secondary structure with the codon in the loop, and it has been mooted that it is some aspect of this structure that is recognised by the *E. coli* ribosome.

This attractive notion seems to be confounded by work by Maizels (see page 647 of this issue of *Nature*). She had already reported the sequence of the first 63 bases of the lactose operon messenger RNA transcribed from the CAP-independent UV5 promoter mutant. The first AUG triplet occurs at positions 39–41 and is followed by 21 bases that code for the first seven amino acids of β -galactosidase. This suggested that translation

might initiate at position 39 and that ribosomes should protect the surrounding region.

Maizels has gone on in her new article to sequence the region of this messenger that is protected by the ribosome in the initiation complex. The results gratifyingly show that the region around position 39 is indeed protected, though the precise length of the protected region depends on the activity and nature of the degrading endonuclease. It next turns out that no obvious secondary structure can be constructed with the codon in the loop.

The next step was to inspect the various ribosome protected regions obtained from the *lac* messenger and from the phages to see if common sequences are present. From this several generalisations emerge. First, there is always a nonsense triplet within 22 bases of the initiator codon, on the 5' side; second, seven of the eleven sequences including the *lac* messenger show the quadruplet AGGA on the 5' side; third, many of the sequences, again including *lac*, show a sequence PuPuUUUXPu (Pu is a purine and X is usually a purine). The significance of these findings is, however, further confused by the fact that these common subsequences do not occur in the same position relative to the initiator codon or even in the same order.

The question remains as to whether these results provide a tantalising glimpse at the requirements for initiator recognition or whether they are only fortuitous; for the possibility is still open that the binding of the ribosome to the protected region is not the primary event and that the recognition involves some other part of the messenger or even that the recognition process involves some other messenger specific factor.

E. G. RICHARDS

tinental regions results in zoning caused by layers which differ in chemical composition". In addition, regional variations in temperature-depth distributions will cause corresponding variations in elastic properties. In spite of these possibilities, however, any or all of which may play an actual part in the formation of the asthenosphere, the consensus is that they are not by themselves sufficient to account for, among other things, a decrease in S wave velocity from about 4.6 km s^{-1} immediately below the lithosphere to 4.3 km s^{-1} (or even 4.1 km s^{-1} in places) at a depth of about 100 km. This is why several workers, beginning with Anderson (*Sci. Am.*, **205**, 2; July 1962), have suggested that the low velocity zone implies a small degree of partial melting—a proposal which goes rather further than an earlier one to the effect that the low velocity part of the mantle is close to the melting point.

Since this suggestion was first made, the conditions under which a small but significant part of the mantle would remain in a state of partial melting have been examined in some detail. It soon became evident, for example, that the stability of a very small degree of partial melting would be difficult to maintain if the mantle were completely anhydrous. As Green (D.H.) and Ringwood have pointed out (*Beitr. Miner. Petrogr.*, **15**, 103; 1967), at pressures of 20–30 kb the melting temperature interval between the solidus and liquidus of olivine tholeiite and picrite is less than 100°C , and within this range dry pyrolite would probably become 30% molten. Even temperature fluctuations as small as 20°C would produce about 5% partial melting. In other words, for a dry mantle the degree of partial melting is very susceptible to temperature changes, making for an instability with which magma bodies would form and surface volcanism would occur on a greater scale than is actually observed. Or to put the matter another way, the assumption that partial melting occurs in the mantle because the geotherm rises above the dry solidus cannot be sustained because the geotherm would have to be finely controlled and constrained close to the solidus to prevent the production of too much liquid and the resulting instability.

So some stabilisation mechanism must be found; and one which has achieved a high degree of creditability in recent years is the presence of water. Numerous experimental studies have shown that even a very small proportion of water (say, 0.1%) will lower the melting temperatures of material thought to comprise the upper mantle by several hundred degrees, thereby increasing the interval between the solidus and the liquidus. The chemistry and mineralogy is quite involved, but the net physical effect of this increase is greater stability against temperature changes. After the occurrence of a very small degree (say 1%) of partial melting—often termed incipient melting to distinguish it from the 5–30% partial melting required to form magmas—a comparatively large change in temperature will cause only a very small change in the degree of melting. And the fact that the amount of liquid produced varies little throughout a wide range of temperatures is widely thought to explain how a low velocity zone with little variation in properties can exist beneath a wide range of tectonic environments.

Details apart, however, what is clear from all this is that a great deal of thought and experimental work has gone into supporting the apparently simple proposition that the asthenosphere is a manifestation of incipient melting. This does not necessarily mean that the conclusion is right, of course—but at the same time, it does mean that anybody coming to the opposite view faces a difficult task in convincing others. In their article on page 617 of this issue of *Nature*, Green (H.W.) and Gueguen come to the opposite view. Whether their argument will be thought to carry the necessary conviction remains to be seen.

In the meantime, it is interesting to note the mild irony involved in the fact that Green and Gueguen come to

their conclusion from a study of kimberlite pipes. The fact that kimberlite pipes (which occur in India, the United States and Siberia but are best known over about two million square kilometres of southern Africa) contain diamonds implies that the kimberlite magma originated at asthenospheric depths (pressures), or, as Green and Gueguen themselves argue, at lower asthenospheric depths. And the fact that they contain large numbers of xenoliths (genetically unrelated inclusions) acquired on the upward journey, not only of crustal rocks but also of material not known to occur in the local crust, implies that the 'unknown' xenoliths comprise a random sample of upper mantle rock types cut by the pipes. So the xenoliths are a valuable source of information on the composition of upper mantle materials (at least beneath stable continental regions)—materials whose studied behaviour led to the conclusion that the asthenosphere must be partially melted. But now Green and Gueguen have used the very same xenoliths (among other things) in quite a different way to show that the asthenosphere cannot be partially melted.

PETER J. SMITH

Magnetic superfluid

THE observation by Bozler, Bernier, Gully, Richardson and Lee of Cornell University (*Phys. Rev. Lett.*, **32**, 875; 1974) of a longitudinal nuclear magnetic resonance at the frequency which had been predicted by Leggett of the University of Sussex (*ibid.*, **31**, 352; 1973) suggests that theory is again starting to catch up with experiment in attempting to reach an understanding of superfluid ^3He .

For more than a decade theory had been well ahead in that a superfluid transition had been predicted, but had not been discovered experimentally. This prediction stemmed from an extension of the theory which Bardeen, Cooper and Schrieffer had devised (the BCS theory) to account for superfluidity of the electron gas in a superconducting metal, to describe assemblies of neutral particles, and liquid ^3He in particular. The basis of the BCS theory is the idea that, in the presence of an attractive interaction between themselves, the electrons can reduce their total energy by forming pairs with opposite spin and momentum. To break apart one of these co-called Cooper pairs requires a certain minimum amount of energy, so that any dissipative process which is unable to provide this minimum energy cannot occur, and flow of the electrons without dissipation of energy is therefore possible provided that a critical velocity is not exceeded. In the case of a superconductor the attractive interaction between the electrons, which is able to overcome their mutual Coulomb repulsion, arises through slight distortions in the lattice of positive ions in the region of each electron.

Like electrons, ^3He atoms also carry a net spin angular momentum of $\frac{1}{2}h$ and similarly, being fermions (unlike ^4He atoms), it is rigorously forbidden for more than one of them to be in the same state. Liquid ^3He is, of course, in other ways very different from a superconductor. In particular, there is no lattice and the atoms of which it is composed are electrically neutral. It was realised, however, that an effective attractive interaction might exist between two ^3He atoms if their mutual angular momentum was nonzero, so that a new form of Cooper pair might thus be created. This orbital angular momentum had to be quantised as lh , with l an integer, and a variety of suggestions was made concerning both the likely value of l and also the relative orientations of the two spins and the orbital motion of the pair which would give the lowest energy, and thus represent the most probable state of the assembly. Because such an assembly is composed of neutral atoms, the predicted state was referred to as a 'p-wave' superfluid rather than superconducting.

The observation in 1972 by Osheroff, a postgraduate

student at Cornell, that liquid ^3He under pressure undergoes two separate phase transitions as it is cooled below 3 mK seemed to indicate that the long predicted superfluid state had been discovered. Subsequent experiments at Cornell, La Jolla and Helsinki seem to have confirmed that both new phases of the liquid are indeed superfluids.

When a magnetic field is applied to normal liquid ^3He , above the superfluid transition temperatures, each nuclear spin has a choice of occupying one of two possible states separated by an energy $h\omega_L$, depending on whether it is parallel or anti-parallel to the field. The absorption of energy which occurs when electromagnetic radiation of frequency ω_L is applied at right angles to the magnetic field, is referred to as nuclear magnetic resonance (NMR), and is a consequence of transitions being induced between the two energy levels. It was found that as liquid ^3He cooled below the higher temperature phase transition into the so called A phase, the NMR frequency rose rapidly; and on reaching the second transition and entering the lower temperature B phase, the frequency suddenly reverted to the original value which is possessed in normal liquid ^3He . From these phenomena it was inferred that Cooper pairs in the A phase were formed with their spins parallel, producing a resultant spin angular momentum with consequent magnetic effects; but that those in the B phase were probably antiparallel, with no resultant spin and therefore no shift in the NMR frequency. It seems probable that in both the superfluid phases the orbital action of all the pairs is highly correlated so that there is a resultant orbital angular momentum describing the liquid as a whole, which might therefore be regarded as an orbital ferromagnet, and many of whose properties would clearly vary depending on the direction in which they were measured. An anisotropic superfluid of this sort is an object previously unknown in nature.

Much theoretical discussion has centred on the way in which the spins and orbital angular momenta are correlated with each other, but it now seems fairly well agreed that in the A phase the Cooper pairs each have a resultant orbital angular momentum of magnitude lh , and that the spin and orbital moments for each pair lie at an angle to each other. Leggett, in his 1973 paper, considered various states of this type and predicted the magnetic properties which might be observed for each. In particular, he showed that for one possible state, the so called axial state, there should be a longitudinal NMR absorption, in addition to the usual transverse one, whose frequency $\omega(T)$ was independent of magnetic

field and which was connected by the simple relation $\omega^2 = \omega_L^2 + \omega_0^2(T)$ to the shift with temperature of the transverse NMR signal of frequency ω .

Members of the Cornell group have now carried out the experiment suggested by Leggett. They have been able to determine $\omega_0(T)$, first, by observing directly the predicted longitudinal resonance and, second, by measuring the shift with temperature of the transverse resonance. Values of $\omega_0(T)$ derived in these two different ways were indeed found to be in good agreement with each other, except very close to the upper transition temperature. Many features of the data require further investigation, notably the change with temperature of the line-width of the longitudinal resonance, but the Cornell experiment does appear to have demonstrated unambiguously that the A phase of superfluid ^3He is in the axial state described by Leggett.

From a Correspondent.

Hexosaminidase and Tay-Sachs disease

from a Correspondent

ONE of the two main electrophoretically distinguishable N-acetyl hexosaminidases (hexosaminidase A) is deficient in tissues of patients with Tay-Sachs disease, a fatal inherited disorder of infants in which the complex lipid, ganglioside GM_2 accumulates excessively in the brain. Hydrolysis of the terminal N-acetyl galactosamine from GM_2 is presumably normally catalysed by hexosaminidase A but not hexosaminidase B. Several important questions still remain to be answered, however, concerning the nature of the relation of the enzymes to each other and also the low activity reported (Sandhoff and Wässle, *Hoppe-Seyler's Z. physiol. Chem.*, **352**, 1119; 1971) of purified hexosaminidase A towards GM_2 .

That a genetic relationship exists between A and B was indicated by the discovery that in some patients both the A and B enzymes are deficient (Sandhoff's disease). The enzymes also resemble each closely—in particular they have the same molecular size, and antisera raised to either enzyme will cross react with the other form. Two main hypotheses have been seriously considered, one being that A is formed from B by an enzymatic conversion which affects a non-protein portion of the molecule (the converting enzyme being deficient in Tay-Sachs disease) and the other that the enzymes are both multimeric being made up of similar and dissimilar subunits.

These problems have been discussed again recently in several papers and

some interesting new evidence has been presented (Srivastava *et al.*, *J. biol. Chem.*, **249**, 2043; 1974; Srivastava *et al. ibid.*, 2049; Srivastava and Beutler, *ibid.*, 2054; Lalley *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1569; 1974; Li *et al.*, *J. biol. Chem.*, **248**, 7512; 1973). Srivastava, Beutler and colleagues have purified the N-acetyl hexosaminidases of human placenta to homogeneity. They claim that the amino acid compositions of the two enzymes are different. The molecules were found to be multimeric by SDS (sodium dodecyl sulphate) electrophoresis (and if they are right they are hexamers). Immunological studies have demonstrated that a specific anti-A antibody can be formed in addition to the non-specific cross reacting anti A/anti B. Patients with Tay-Sachs disease possessed no A antigens whereas one of the two 'Sandhoff' patients had antigenically detectable A and B proteins, and the other patient had the A protein only. The authors conclude that the A and B enzymes possess subunits in common (β) and that at least A must have specific subunits (α) determined by a separate locus such that A is $(\alpha\beta)_n$ and B is $(\beta\beta)_n$ or alternatively $(\beta\gamma)_n$ (γ representing a polypeptide subunit determined by a third locus). The defect in Tay-Sachs disease would represent a mutation causing the diminished production of α chains, whereas that in Sandhoff's disease would be a mutation of the β chain (the two patients described probably having different β mutations).

Suggestive evidence for this type of model came from the immunological findings, but the main support came from the amino acid analysis so it will be of particular interest to see whether the amino acid differences (which are certainly not great) can be substantiated.

Evidence which is consistent with the two locus model of Srivastava *et al.* (and also with the conversion model) is presented by Lalley *et al.* from their human-mouse cell hybrid results. As the human chromosomes are shed from hybrid cells the enzymes determined by genes on those chromosomes are also lost. On careful examination of 60 hybrid clones they observed the loss of A and B together, A without B, but never B without A, and concluded that the expression of A requires the presence of the gene coding for B as well as the gene for A expression, and that the two loci are on different chromosomes. They also provide a probable explanation for the conflicting results of another group (van Someren and van Henegouwen, *Humangenetik*, **18**, 171; 1973).

Synthetic substrates have largely been used to characterise the hexosaminidases. Srivastava *et al.* and also Li *et al.* report that much of the original activity

of their tissue extracts towards the presumed *in vivo* substrate GM₂ was lost on purification of hexosaminidase A. Li and colleagues, however, provide exciting new evidence that hexosaminidase A (but not B) activity towards GM₂ can be stimulated by a non-dialysable, non-enzymatic, heat-stable liver 'factor'. How does this finding fit in with the subunit model? Is the factor ever an integral part of the hexosaminidase A molecule? Further purification and characterisation of this component will clearly be informative.

East and West ribosomes

from a Correspondent

A SYMPOSIUM on ribosomes organised at Schloss Reinhardsbrunn from May 13–16 by the Biochemical Society of the German Democratic Republic provided a rare opportunity for scientists from East and West to discuss their work together at first hand. Many of the reports came from the two big ribosome groups in Berlin, with eukaryotes to the East of the Wall and prokaryotes to the West, and the time of the meeting was evenly divided between the two classes of ribosome.

To start with the prokaryotes, H. G. Wittmann summarised the work being done in West Berlin on mutant proteins from both subparticles of *Escherichia coli* and B. Wittmann-Liebold (W. Berlin) described amino acid sequences of many of these proteins; a clear pattern of amino acid changes in mutants of several proteins has emerged. The emphasis in *E. coli* has moved to the 50S particle, and R. Brimacombe (W. Berlin) reported two 50S ribonucleoprotein fragments, one containing proteins known to associate with 5S RNA. From the work of V. Erdmann (W. Berlin), it is now clear that 5S RNA is capable of interacting with the T ψ CG loop of transfer RNA, and D. Richter (W. Berlin) showed further that oligonucleotide T ψ CG inhibits ribosomal functions associated with the A (aminoacyl tRNA) site, but does not affect the P (peptidyl tRNA) site. I. Rychlik (Prague) described a very specific inhibitor (TPCK) which prevents the formation of a ternary complex between tRNA, GTP and the elongation factor T_u. In other experiments on 50S sites, K. Nierhaus (W. Berlin) rebound single 50S proteins to lithium chloride cores, to identify the proteins involved in peptidyl transferase activity and chloramphenicol binding. A similar approach was used by G. Howard (Basel Institute for Immunology) to look for proteins involved in binding of elongation factor G and the antibiotic thiostrepton.

Affinity labelling is a technique

which is being used with great success to pinpoint functional centres on the ribosome. O. Pongs (West Berlin) reported experiments with analogues of chloramphenicol and puromycin, and W. Möller (Leiden) an analogue of GTP. E. Kuchler (Vienna) used affinity label analogues of both tRNA and mRNA designed to attach to protein, and also a tRNA analogue which reacted with the 3' half of 23S RNA. A. Girshovitch (Poustchino) and D. Knorre (Novosibirsk) both preferred to use tRNA analogues which could attach to protein or RNA, and found that the RNA was preferentially attacked.

Coming now to the eukaryotic results, the affinity label method has also been used successfully here by J. Stahl (E. Berlin) to identify two proteins at the 60S A site with a puromycin analogue. H. Welfle (E. Berlin) and P. Westermann (E. Berlin) reported experiments on iodination of rat liver ribosomes. Those of Westermann were particularly interesting, as he was able to show which proteins are specifically protected from iodination by bound mRNA, tRNA and factors. W. Möller (Leiden) found that two acidic proteins from yeast 60S ribosomes could be interchanged with proteins L7/L12 from *E. coli*, with retention of EF activity. The surface of eukaryotic ribosomes is rich in pyrimidines, according to M. Saarna (Tartu) who suggested that these could interact with poly(A) as the first step in mRNA binding.

On the strictly functional side, H. Bielka (E. Berlin) showed that ribosomes from different tissues are very similar in activity, but that the corresponding cytosolic fractions are very variable. The ever-increasing complexity of mammalian initiation was summarised by T. Staehelin (Basel Institute for Immunology); the number of initiation factors now stands at six, and ATP is also involved. H. Bloemendahl (University of Nijmegen) described his calf eye-lens cell-free system, which seems a useful alternative to the reticulocyte system, and a good summary of current knowledge of ribosome-membrane interaction came from G. Blobel (New York). G. Shapira (Paris) talked about his new RNA species which stimulates protein synthesis; it is about half the size of tRNA, and seems not to be interchangeable between different systems.

Finally, the electron microscopists V. Vasiliev (Poustchino), N. Kiselev (Moscow) and G. Lutsch (E. Berlin) were in reasonable agreement that the smaller subparticle of both pro- and eukaryotic ribosomes is divided into two distinct regions of unequal size, but failed to agree on the shape of the large subparticle of eukaryotes.

Protein receptors of juvenile hormone

from our
Insect Physiology Correspondent

It has long been assumed that the lipid soluble juvenile hormone must be transported in the haemolymph in protective association with a hydrophilic carrier which was likely to be a protein. Emmerich and Hartmann (*J. insect Physiol.*, **19**, 1663; 1973) have found that in the haemolymph of adult female *Locusta* there are six distinct lipoprotein fractions and one of these (molecular weight 220,000) binds the juvenile hormone of *cecropia*. It closely resembles the lipoprotein which binds juvenile hormone in *Hyalophora cecropia* itself (Whitmore and Gilbert (*J. insect Physiol.*, **18**, 1153; 1972)). It seemed a reasonable suggestion that this might in fact be a specific carrier lipoprotein which could have a very important role in the action of the juvenile hormone in preventing its unspecific incorporation into the lipophilic phases of the cells and its rapid breakdown to inactive carboxylic and hydrated products.

But Kramer *et al.*, with Law (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 493; 1974), have now recognised the existence of a somewhat different specific juvenile hormone binding protein in the haemolymph of the hawkmoth caterpillar *Manduca*. The authors point out that the C₁₈ juvenile hormone, the chief form in the silkworm *Hyalophora cecropia*, has considerable solubility in water, yielding a monomeric solution exceeding 10⁻⁵M. The binding protein has a molecular weight around 34,000; it has a much higher affinity for the hormone and its analogues than for the products of hydrolysis. Kramer *et al.* found that there is sufficient of this specific protein carrier in the haemolymph and it binds hormone at a sufficiently low dissociation constant, for it to take up virtually all hormone present in normal physiological conditions. They find, indeed, that the juvenile hormone is taken up by lipoprotein only when the binding protein has been saturated.

Besides transport in the haemolymph, the question arises as to whether, in analogy with the action of oestrogens in vertebrates, the juvenile hormone binds to a specific receptor in its target organs. It is well known that the insect epidermis is the chief site of action of the juvenile hormone. Schmialek *et al.* (*Z. Naturforsch.*, **28C**, 173 and 453; 1973) have obtained new evidence of a receptor protein in the epidermal cells of the *Tenebrio* pupa. Using the juvenile hormone analogue 10, 11-epoxy-6, 7-trans-2, 3-trans-farnesylpropenylether as the active substance, they found that the target cells will accumulate this against

a concentration gradient and will degrade it far more slowly than non-target organs. The receptor has a molecular weight of 360,000 and seems to be a unique compound. It has a very high affinity for the active substance, a very low affinity for the breakdown products. It contains RNA in association with protein.

Filling the gap with the pin cherry

from Peter D. Moore
Plant Ecology Correspondent

It is now fairly well established that during the course of succession leading to deciduous forest in temperate climates, the peak in plant species diversity occurs at about 100–200 years (Loucks, *Am. Zool.*, **10**, 17; 1970). Following this the full attainment of dominance by deep shade-casting trees and the establishment of a shade tolerant understorey community lead to the elimination of many light demanding plant species more typical of the seral stages. Natural climax forest, however, has an uneven population age structure and is subject to random perturbations resulting in the opening of the canopy, for example as a consequence of lightning strikes and wind-blows. A mature, stable forest in equilibrium with its environment possesses fragments of seral vegetation within its clearings, and its species diversity is therefore greater than one might suppose, if an adequate sample area is studied (see Hope Jones, *Br. Birds*, **65**, 291; 1972).

Marks (*Ecol. Monogr.*, **44**, 73; 1974) has made specific studies of these self-healing attributes of climax ecosystems. In particular he has concentrated on the modifications in life cycle, and dormancy and seed dispersal mechanisms associated with those species which recolonise areas laid bare by natural perturbations. In ecosystem terms, a gap represents a suboptimal utilisation of the available resources—for example, light, moisture, nutrients—and the species which are to exploit such a situation must either arrive rapidly by seed, or their dormant seeds already present in the soil must germinate and grow.

In New Hampshire, an important species which fills this role is the pin cherry (*Prunus pensylvanica*). It is not found in mature forest communities, but is restricted to clearings in which the forest has suffered disturbance. If the soil of a disturbed area contains abundant pin cherry seeds in a viable state, then a dense stand with a closed canopy develops within about 3 yr. In this case the climax species, sugar maple (*Acer saccharum*) and beech

(*Fagus grandifolia*) grow beneath the canopy and assume dominance after about 25–35 yr. If the original soil density of pin cherry seeds is low, then birch and aspen become established at an early stage by seed coming from outside the system, and the final dominance of sugar maple and beech may be delayed until 80–90 yr have passed.

This buried seed strategy which results in the maintenance of pin cherry populations, demands a fairly high density of viable seeds surviving in the soil of the mature phase of the forest. Marks found densities varying between 300,000 and 500,000 viable seeds per hectare at two such mature forest sites in which pin cherry was rare or absent. Original fruit production by dense pin cherry stands was estimated to be between 2 and 3×10^6 fruits ha⁻¹. Germination is triggered by forest disturbance; in laboratory tests removal of endocarps stimulated germination. The longevity of seeds in soil is difficult to examine experimentally, but was estimated to be about 50 yr. The clearance of forest which had been in mature phase for 80–100 yr resulted in little pin cherry regeneration.

Once established, pin cherry grows and accumulates nutrients more rapidly than the final climax dominants, which accounts for its success during the early stages of forest recovery. As far as ecosystem regrowth is concerned, the nutrient demand of pin cherry results in the conservation of nutrient capital which might otherwise have been lost by leaching.

Stability in climax ecosystems is usually defined in terms of the system's capacity to recover from disruption. Evidently this stability depends ultimately on species, like the pin cherry, which may be totally absent from stands of mature climax dominants, or represented only by seeds lying dormant in the soil.

Polyadenylation in the cytoplasm

from Benjamin Lewin
Molecular Genetics Correspondent

THE purpose of nuclear polyadenylation remains obscure and an addition to this puzzle is now made by the observation of Slater and Slater (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 1103–1107; 1974) that cytoplasmic polyadenylation takes place in sea urchin eggs. It is by now well established that in mammalian cells a sequence of about 200 adenine residues is added to the 3' end of an heterogeneous nuclear (hn) RNA molecule before cleavage of this region. The poly(A)-containing sequence is then transported to the cytoplasm to serve as messenger RNA. Addition of poly(A)

takes place only in the nucleus of mammalian cells; since inhibiting the addition of poly(A) prevents processing of mRNA, it seems reasonable to suppose that poly(A) is implicated in some essential way in the maturation process.

But poly(A) is also present in viral messengers found only in the cytoplasm and in mitochondrial messengers. Speculation that it may therefore also have a direct role in translation seems to have been refuted by research in several laboratories, which has shown that mRNA lacking poly(A) can be translated as efficiently as mRNA containing it. But force to the argument that nevertheless poly(A) must be implicated in some way in the cytoplasmic function of mRNA is now lent by the results that Slater and Slater report.

Fertilisation of sea urchin eggs causes a sudden burst of synthesis of poly(A); the recent experiments of Slater *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 406–411; 1973) and Wilt (*ibid.*, 2345–2349) have suggested that this might represent the addition of poly(A) to previously existing messengers, a situation which contrasts with the addition of poly(A) only to newly synthesised nuclear transcripts in mammalian cells. Slater and Slater now report experiments to test this model. By incubating fertilised eggs with both ³H-adenosine and ¹⁴C-uridine, they distinguished newly synthesised mRNA (which possesses the ¹⁴C label) from mRNA synthesised before fertilisation (which lacks this label). All RNA possessing poly(A) should contain a tritium label; and if this mRNA was synthesised previously the ³H-adenosine should be located exclusively in the poly(A) segment, whereas if these molecules are newly synthesised some labelled adenosine should be present also in the other parts of the messenger.

The ³H and ¹⁴C labels show a different sedimentation distribution, which implies that they must enter different mRNA species; that is, the poly(A) is not added simply to newly synthesised messengers. The ³H-adenosine which enters the mRNA fraction is found very largely in the poly(A) fraction which can be reclaimed after digestion with ribonuclease; this suggests that only the poly(A) part of the molecule is newly synthesised.

This implies that some mechanism must exist in the cytoplasm for polyadenylating messenger RNA, a conclusion which raises many important questions about the possible role of this polyadenylation. One obvious question is whether the messengers to which poly(A) is added after fertilisation possessed any poly(A) to start with or whether they completely lacked it. If messengers synthesised before fertilisation lack poly(A), then they must be produced by a mechanism in part different from that prevailing when

poly(A) is added at the nuclear stage. Similar questions to those asked about the nuclear addition of poly(A) can be directed to the cytoplasmic addition—are only certain messengers selected for polyadenylation, what happens to messengers which do not receive poly(A), does polyadenylation have some role in controlling translation? No function for poly(A) in the cytoplasm is yet apparent, but with teleological arguments becoming ever more popular in molecular and cell biology, many will be tempted to conclude that some important function of the poly(A) remains to be discovered.

Eliminating Doppler broadening

from a Correspondent

THE spectra of atoms (and molecules) are replete with information some of which is unfortunately inaccessible because the fine structure of spectral lines is often unresolved. Consequently, spectroscopists strive constantly to improve their resolution. The limitations imposed on spectral resolution are not, however, always instrumental but can be inherent in the atomic assembly. For example, atoms of a gas present a whole range of velocities to the direction of propagation of a light source because of their thermal motions. Now if ν_0 is the frequency of radiation required to raise an atom from its (sharp) lower state to a (sharp) upper energy state when it is stationary relative to the source, then an atom receding from the source 'sees' a number of waves per second (that is a frequency) less than ν_0 . The receding atom must, of course, absorb radiation which it perceives to have frequency ν_0 and which relative to the stationary source must therefore exceed ν_0 . That the Maxwell-Boltzmann distribution of atomic velocities in the source direction ensures a distribution of absorption frequencies even though each atom has sharp energy levels—the so-called Doppler broadening.

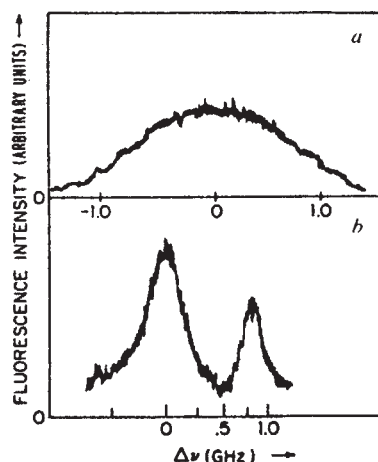
Doppler broadening can be overcome if one selects only atoms within a narrow band of velocities relative to the source so that they all absorb at the same frequency. Several velocity selection techniques are used, including atomic beams and laser saturation spectroscopy (see *Nature*, **235**, 127; 1972). A further, elegant method of dealing with Doppler broadening is now described independently by two groups (Biraben, Cagnac and Grynberg, *Phys. Rev. Lett.*, **23**, 643; 1974; Levenson and Bloembergen, *ibid.*, 645).

The essence of the technique used by these authors is very simple. Both groups investigate the normally forbidden $5S \leftarrow 3S$ electronic transition of the sodium atom by means of two-

photon absorption. This transition is, in fact, a closely-spaced doublet (because of coupling of electronic and nuclear spin angular momentum) with separation 784 MHz but its resolution is precluded in the presence of the aforementioned Doppler broadening which confers a half-width of about 1,000 MHz on each component.

Low pressure sodium vapour is subjected to monochromatic radiation from a tunable laser at a frequency equal to one half that of the $5S \leftarrow 3S$ transition but corresponding to no other known energy state separation within the atom. Those few sodium atoms of thermal velocity such that the $5S \leftarrow 3S$ level separation is Doppler-shifted into resonance with twice the laser frequency can absorb simultaneously (but with low probability) two photons from the radiation and so raise atoms to the $5S$ state. As the laser is tuned, a weak Doppler broadened absorption profile (detected by counting photons emitted from the $5S$ state) is generated as shown in the figure (a). When, however, a mirror is positioned so as to reflect the radiation back through the sodium vapour and establish within it a radiation standing wave composed of two oppositely travelling waves, a given atom can absorb two photons by an additional mechanism—one from each of the counter-propagating beams.

It is clear therefore that for any atom the Doppler shift associated with absorption of a photon from one beam is equal and opposite to that associated with absorption from the other beam. The net result is that each and every atom, regardless of its velocity relative to the radiation source, absorbs two photons from the standing wave but only when the laser frequency is exactly one half the $5S \leftarrow 3S$ level separation. When the laser is tuned through this frequency the doublet character of the transition is resolved (see figure (b)), the Doppler broadening having been neatly eliminated.



One expects the weak Doppler broadened signal already mentioned as arising from absorption of two photons from one of the component travelling waves to provide a background pedestal to the observed doublet of figure (b), but this has been eliminated by an additional sophistication of the experiment. The two oppositely travelling beams are arranged to be circularly polarised in opposite senses. A rigorous selection rule governing transitions between atomic electron states precludes the $5S \leftarrow 3S$ transition if both photons are absorbed from a single beam of radiation circularly polarised in only one sense but not if one photon is absorbed from each of two beams of opposite circular polarisation. The weak Doppler broadened pedestal is therefore excluded while the sharp doublet remains.

Little evidence for Palaeozoic arthropod and plant interaction

from Barry Cox
Vertebrate Palaeontology Correspondent

ON April 30, the Palaeobotanical Group of the Linnean Society of London organised a symposium on the interrelationships of Palaeozoic terrestrial arthropods and plants, which took place at the society's rooms in Burlington House. Several contributions set the Palaeozoic scene. M. A. Calver (University of Leeds) discussed the depositional environment of the British Coal Measures. He showed the gradually decreasing frequency of the marine incursions into this low-lying area of coastal swamps, and briefly reviewed the two conflicting theories of the deposition of coal. R. H. Wagner (University of Sheffield) described the distribution of the Upper Carboniferous flora of the world. He contrasted the rich equatorial flora of Euramerica with the poorer and more conservative Gondwana flora, and showed that there were some indications of different swampland, dryland and upland floras.

Turning to the vertebrates, A. L. Panchen (University of Newcastle upon Tyne) described the ecology and distribution of Carboniferous tetrapods, and suggested that the tetrapods might have evolved in Euramerica. Though herbivorous reptiles probably evolved in the Upper Carboniferous, their remains are rare until the Lower Permian.

The general evolutionary background of the arthropod radiation was described by S. M. Manton (Queen Mary College, London). W. D. I. Rolfe (University of Glasgow) dealt with the

non-insectan arthropods of the Palaeozoic. The most spectacular of these were the 6-foot long arthropleuroids of the Carboniferous of Euramerica, which seem to have lived within the leaf litter, splitting it apart with their flattened heads and bodies—and their gut contents suggest that they fed on lycopod material. The Palaeozoic mites seem to be related to living forms which feed on fungi.

The biology of Upper Palaeozoic insects was discussed by R. J. Wootton (University of Exeter). Insects were almost unknown before the Upper Carboniferous and, even then, knowledge of them is almost exclusively limited to the Euramerica coal swamp fauna. The modern herbivorous groups of insect were almost unknown in the Palaeozoic and holometaboly, which enables totally different adaptations of larva and adult, did not appear until the Permian. Such forms as *Stenodictya* may have been a sap sucker or have picked up spores from sporangia.

Finally, two contributors dealt specifically with the theme of this symposium. W. G. Chaloner (Birkbeck College, London) produced some possible (if contentious) examples of possible plant-animal interaction. These included some plants from the Rhynie Chert which appear to have been damaged during life, and the strong coats of some Devonian spores which may have been adjusted to resist chewing or digestion. P. D. W. Barnard (University of Reading) categorised the ways in which animals could have used plants as food, and the types of evidence of this activity that might have survived. The instances that he had been able to gather together, however, were few. Plumstead has described leaves of *Glossopteris* with ragged edges which could well have been caused by browsing arthropods. Holes in the seeds of *Samaropsis* have been described by Sharon, and Barnard himself has described holes in the nut-like megaspore of *Trigonocarpus*; both of these could well have been due to attack by insects. Finally, some coprolites contain spores, and a specimen of the reptile *Protorosaurus* contains conifer seeds in its abdominal region.

Despite Barnard's best efforts, one's prevailing impression of the symposium was of a production of Hamlet in which the Prince made only fleeting appearances, and indeed there seems little chance of any great variety of unequivocal evidence of the interaction between Palaeozoic plants and arthropods. The meeting also served to demonstrate what a high proportion of knowledge of Carboniferous life is derived from one single (and possibly unusual) environment—the swamplands of Euramerica.

Why do pions eject 'alpha particles' from nuclei?

from Peter E. Hodgson
Nuclear Theory Correspondent

SEVERAL recent investigations have shown that when slow pions or kaons are captured by nuclei, the particles emitted promptly are very often such that the residual nucleus is an excited state of the target less one, two or more α particles or the equivalent numbers of neutrons and protons. The most plausible explanation is that somehow the pions or kaons eject α particles but this has not yet been established because the residual nucleus is identified only by the γ rays it emits, and the particles emitted previously are not detected.

A further important result has been obtained by V. G. Lind and colleagues working in the United States (*Phys. Rev. Lett.*, **32**, 479; 1974), who looked at the results of interactions of fast negative pions (220 MeV) with a range of light nuclei from carbon to vanadium. It is very difficult to detect directly the particles emitted from these intersections so, as in the previous work, the prompt γ rays were studied instead. These are emitted immediately after the particles resulting from the initial interaction. Some of the γ -ray spectra are shown in the figure, and it is notable that there are several peaks common to the spectra from different nuclei.

The energies of these γ rays identify the nucleus from which they were emitted, and it is then found that in the case of even-even targets the most likely residual nuclei correspond to removing one, two or several α particles (or the equivalent numbers of nucleons) from the target. In the case of the odd-*A*

nuclei the γ rays correspond to the removal of a triton or an α particle or a triton plus an α particle from the target nucleus. The cross sections for these processes are comparable to the inelastic scattering cross sections. It is found that the proportion of multiple emissions of α particles compared with single emissions increases with nuclear size.

The experimental technique does not distinguish between the emission of an α particle and the emission of two neutrons and two protons, since only the residual nucleus is identified. The relative likelihood of the two processes, and indeed of all possible processes and hence residual nuclei, can be calculated from statistical theory. It is found that this gives results completely different from those found experimentally so that multiple ' α ' emission cannot be explained by the statistical theory.

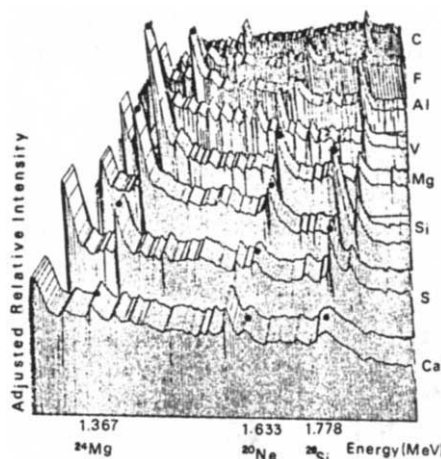
These results have now been confirmed by studies of the interactions of both positive and negative pions with ^{27}Al and ^{28}Si by Ashery and colleagues (*Phys. Rev. Lett.*, **32**, 943; 1974) from Tel Aviv. The negative pions had an energy of 70 MeV and the positive pions energies of 25, 70 and 100 MeV.

Using essentially the same method as the United States group, they also found that there is a large cross section for removing an ' α particle' and leaving the nucleus in its first excited state. This occurs even below 25 MeV, which is the threshold for the emission of two protons and two neutrons, and this strongly supports the hypothesis of cluster emission.

It is found that at 70 MeV the cross section for ' α ' emission is very similar to that for the elastic scattering of pions by free α particles, thus suggesting that the pions are simply knocking out pre-existing α particles from the target nuclei. Substantial cross sections are also found for reactions corresponding to the emission of deuteron clusters.

These experiments on the interaction of fast pions with nuclei thus give results similar to those found in studies of the interaction of slow pions or kaons. There seems to be some process that takes place in the initial stages of the interaction that leads to the preferential emission of α particles. This in turn suggests that there are α particles in the nuclear surface, a hypothesis for which there is already much evidence.

It is important to continue this work, and in particular to try to detect and identify directly the particles emitted in the early stages of the interaction. This is a difficult experimental problem but if it can be solved it will shed new light on the problem of α particles on the nuclear surface.



Some of the prompt γ -ray spectra emitted after interaction of 220 MeV negative pions with a range of nuclei. The lines marked with a solid dot appear in many spectra and correspond to the multiple removal of α particles.

Origin of kimberlite pipes by diapiric upwelling in the upper mantle

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A diapiric mechanism is presented to explain geochemical data on the thermal structure of the upper mantle. It is emphasised that any model to fit the data cannot be a steady state model, and it is concluded that the data are inconsistent with the popular interpretation of the seismic low velocity zone as a partially melted region.

THE 'pyroxene geotherm' technique¹⁻³ utilises the experimentally determined partitioning of calcium⁴ and aluminium² between coexisting clinopyroxene and orthopyroxenes in order to determine the conditions of origin of garnet and spinel peridotites. Application of the technique to xenoliths from kimberlite pipes shows that: (1) the xenoliths are fragments of the upper mantle accidentally included in the kimberlite during its passage to the surface; (2) the xenoliths from each pipe describe a 'palaeogeotherm' which abruptly steepens at depths between 140 and 200 km (Fig. 1); (3) the point of inflection in each geotherm corresponds to chemical⁵ and textural⁶⁻⁸ changes. The shallower, 'normal' portion of the geotherm is characterised by coarsely crystalline harzburgites which have no significant preferred orientation and are strongly depleted in basaltic constituents; the steepened, deeper portion is represented by highly deformed, extensively recrystallised lherzolites capable of producing normal basalts by partial melting⁹. It has been pointed out^{1,8,10} that the shape of the palaeogeotherms is unlikely to reflect a steady state situation and it has been proposed that the steepening results from shear heating which occurred in the seismic high attenuation, low velocity zone (HALVZ) during the breakup of Gondwanaland. We agree that the assumptions necessary for any steady state model are unreasonable but we question that the shear-heating model quantitatively satisfies the data. This article presents an alternative diapiric model, postulating upwelling from within or below the HALVZ.

The model

We assume that a small density inversion of unknown origin initiates upwelling at a depth of more than 200 km. The upwelling will be self-perpetuating if the temperature gradient exceeds that for adiabatic expansion, a well known property of modern models of the thermal structure of the upper mantle¹¹. The upwelling therefore becomes a diapir which very soon reaches a quasi-steady limit velocity. It is driven principally by the buoyancy force which results from the temperature difference between the interior of the diapir and the surrounding, undisturbed mantle. If the velocity of ascent is rapid compared with the rate of heat loss, the diapir may reach the solidus and partially melt. We assume that such melting occurs just below the point of the deepest xenoliths and that the kimberlitic magma is produced. The magma brings up fragments both from the top of the diapir, and from above it.

For quantitative comparison of this model with the geochemical data, the geothermal gradient along the axis of the

diapir can be calculated (Fig. 2). The heat flow equation must therefore be solved. Neglecting heat sources¹²:

$$\rho c_p (\partial T / \partial t + v_i \nabla_i T) = K \nabla^2 T + (\eta/2) (\partial v_i / \partial x_k + \partial v_k / \partial x_i)^2 \quad (1)$$

where ρ is the density, c_p the heat capacity at constant pressure, T is temperature, t is time, v_i is the velocity of the moving rocks, K is the thermal conductivity and η the viscosity. The second, third and fourth terms are the contributions of convection, conduction, and viscous heating respectively. The well known difficulty in obtaining an analytical solution to equation (1) leads to a comparison of the contributions of the various terms thus simplifying the problem but remaining within the accuracy of the data. The relative importance of the convection and viscous heating terms is of paramount importance to the form of our solution. We assume that the spatial variation of velocity within the diapir can be satisfactorily approximated by a single average velocity $\langle v_3 \rangle$. We also assume that the

average temperature gradient $\langle \frac{\partial T}{\partial x_3} \rangle$ is of the order of 1°C km^{-1} ; $c_p = 10^7 \text{ erg } ^\circ \text{C}^{-1} \text{ g}^{-1}$; $\rho = 3 \text{ g cm}^{-3}$, $\eta = 10^{21} \text{ poise}$ and that $\langle \frac{\partial v_i}{\partial x_i} \rangle = \frac{\langle v_3 \rangle}{R}$ where R , the radius of the diapir, is 50 km. For this comparison, we calculate the average velocity by equating the driving force to the viscous drag on the diapir considered as a whole, and using an average temperature

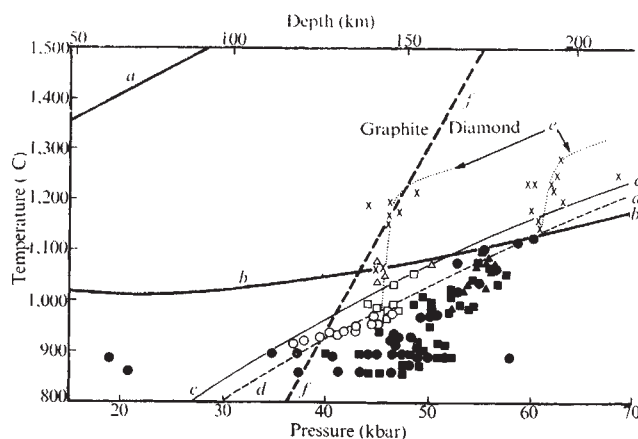


Fig. 1 Thermal and textural structure of the upper mantle beneath southern Africa at the time of emplacement of Cretaceous kimberlite pipes. Data and phase boundaries after Macgregor³: *a*, dry peridotite solidus; *b*, vapour saturated solidus ($\text{H}_2\text{O}:\text{CO}_2 = 1:3$); *c*, C and R shield geotherm; *d*, model geotherm (undisturbed); *e*, model geotherm (perturbed); *f*, graphite-diamond phase boundary. Open symbols, South West Africa region; closed symbols, Kimberley region: circles, coarse grained, undeformed xenoliths with coarse granular textures; squares, coarse grained, undeformed xenoliths with coarse tabular textures; triangles, plastically deformed xenoliths with porphyroclastic textures; crosses, plastically deformed xenoliths with mosaic textures. Shield geotherm of Clark and Ringwood¹¹ (*c*) given for comparison

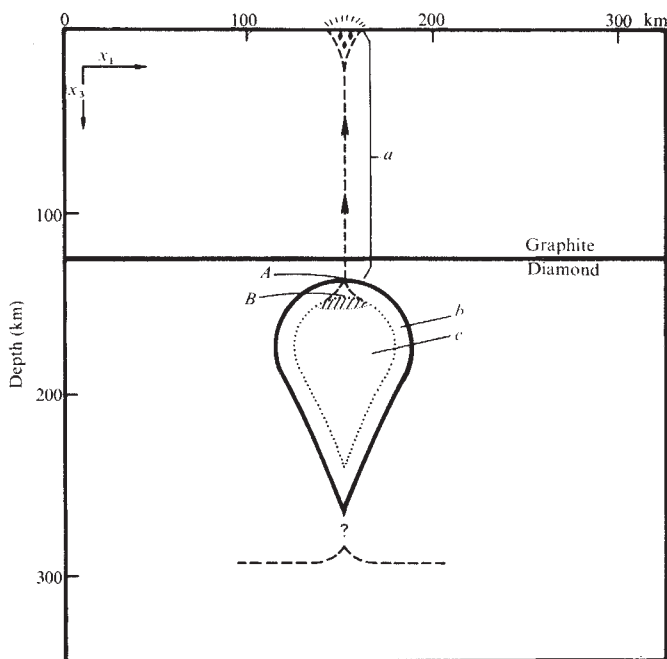


Fig. 2 Idealised drawing of a rising diapir at the time of melting and kimberlite formation. Region *a*, undisturbed lithosphere; region *b*, outer 'rind' of diapir affected by conductive cooling during upwelling; region *c*, interior of diapir cooled only by adiabatic expansion. Points *A* and *B* mark transitions from one region to another. Shaded area diagrammatically indicates melting at the top of region *c*.

difference of 100°C between diapir and surrounding rocks. The result, which is almost independent of the shape of the diapir¹², is $\langle v_3 \rangle = 1\text{ cm yr}^{-1}$. Then:

$$\frac{\rho c_p \langle v_3 \rangle < \partial T / \partial x_3 >}{(\eta/2) (\langle \partial v_3 / \partial x_1 \rangle)^2} \simeq 500 \quad (2)$$

Even for these conservative assumptions, the convection term is dominant by more than two orders of magnitude. The likelihood that η is less than 10^{21} poise further reduces the importance of viscous heating, and therefore the shear heating term cannot have a controlling effect on our model.

A one-dimensional model can be used if the diapir is sufficiently large for heat loss at the sides not to affect appreciably the central axis. For the times relevant to the model, the heat diffusion distance¹² is less than 20 km, so that any diapir large enough not to be affected by shear heating (that is, $R \geq 25\text{ km}$) can also be treated as a one-dimensional problem; the side effects are unimportant in a first-order approximation. Finally, we assume that heat lost through the top of the diapir is carried away by the mantle flowing around the advancing mass. We thus obtain the model shown in Fig. 2. The calculated axial geotherm will have three parts: (*a*) an undisturbed geotherm above the diapir; (*b*) the top of the diapir affected by diffusive loss of heat; (*c*) the tail of the diapir, characterised by the adiabatic gradient. The simplified model matches parts *a* and *c* by a temperature perturbation (*b*) on the model geotherm, which is chosen as a good fit to the geochemical data between 100 and 200 km depth and fitted to the shield geotherm of Clark and Ringwood¹¹ at lesser and greater depths (Figs 1 and 3).

The perturbation obeys the heat flow equation:

$$\partial T / \partial t = (K / \rho c_p) (\partial^2 T / \partial x_3^2) \quad (3)$$

The boundary condition at the top of the diapir is $T_0(t) = v_3 (\partial T / \partial x_3)_0 t$ where t is the time elapsed since the formation of the diapir, $K = 4 \times 10^4\text{ erg cm}^{-1}\text{ s}^{-1}^\circ\text{C}^{-1}$ ($10^{-3}\text{ calorie cm}^{-1}\text{ s}^{-1}^\circ\text{C}^{-1}$) and the other values are as already given. The boundary condition equates the temperature at the top of the diapir with that just above it in the undisturbed mantle; it is the expression of the heat flowing out at the top of the diapir.

Between 250 km and 400 km depth, $(\partial T / \partial x_3)$ is relatively constant and for the calculations we have adopted $1.7^\circ\text{C km}^{-1}$. In this simplified form the problem has the analytical solution¹³:

$$T(x_3, t) = 4 \langle v_3 \rangle (\partial T / \partial x_3)_0 t^2 \text{erf}[x_3 (Kt / \rho c_p)^{-1}] \quad (4)$$

The calculations have been made for the shallowest and deepest data sets of Macgregor³, representing the regions of South West Africa (Louwrensia pipe) and Kimberley (grouped data representing the Wesselton, Roberts Victor, Bulfontein and Jagersfontein pipes) respectively. We have chosen to model the undisturbed mantle with a single, representative geotherm because of the uncertainties in the data, the mixed origin of the Kimberley data, and the small effect separate geotherms would have on our results. The chosen geotherm also fits satisfactorily with the data from the Lesotho region^{1,3}. The data allow the independent calculation of the time intervals of upwelling, the diapir velocities and then the depths of origin (Table 1, Fig. 1). The calculated depths of origin of the diapirs are surprisingly similar and perhaps represent the depth of the lowest portion of the HALVZ.

Discussion

The diapir model fits the geochemical data well and the adjustable parameters in the model are all chosen to be very reasonable values. Similarly, the calculated times and velocities are reasonable. The production of the kimberlite magma containing xenoliths is a natural consequence of the model, for once initiated, the diapir will rise until it reaches the surface without melting (becoming an alpine peridotite¹⁴) or it will partially melt and result in the production of kimberlite pipes. We assume that melting occurs at temperatures just above the hottest temperatures indicated by the analysed xenoliths. These temperatures always fall between the dry and water-saturated peridotite solidi^{15,16}, and the presence of small quantities of water is thus mandatory for the model. The amount cannot be precisely estimated because it is critically dependent on

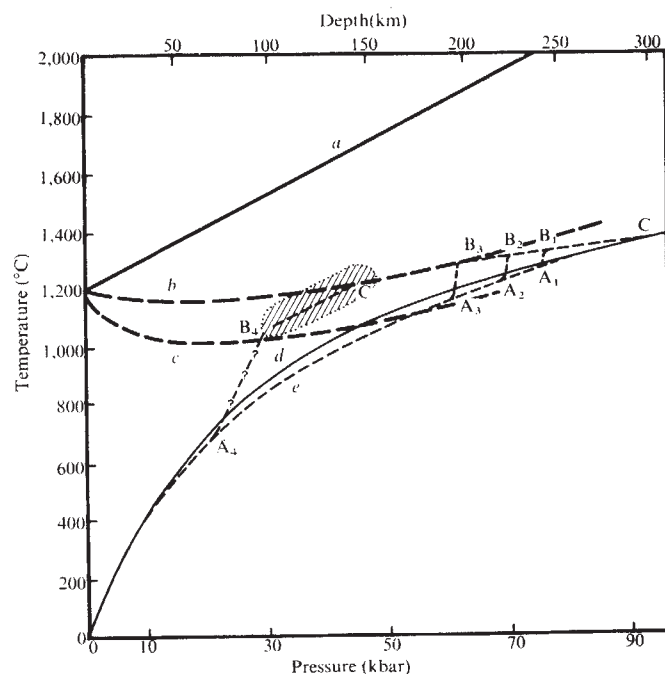


Fig. 3 Thermal evolution of the rising diapir in the Kimberley region. *a*, Dry peridotite solidus; *b*, model solidus; *c*, wet solidus ($\text{H}_2\text{O}:\text{CO}_2 = 1:3$); *d*, C and R shield geotherm; *e*, model geotherm (undisturbed). Point *C* represents origin of the diapir on the undisturbed geotherm. Points *A_i* and *B_i* represent successive positions of points *A* and *B* of Fig. 2; *B* moves down the adiabat and *A* down the geotherm. Melting occurs at *B₃*. Shaded region encompasses data of 'second geotherm' of Macgregor³. *A₄-B₄-C'* represents possible earlier diapir responsible for these data.

Table 1 Duration of upwelling, velocity of ascent, and depth of origin of diapirs

Region	Mean velocity	Duration of upwelling	Original depth
South West Africa	5 cm yr ⁻¹	3 Myr	290 km
Kimberley	3 cm yr ⁻¹	3 Myr	280 km

collection and analysis of xenoliths which equilibrated on the solidus as well as on the accuracy of the pyroxene geotherm. Macgregor³ points out that 'primary phlogopite' is present in region *a* (Fig. 2) of our model. Texturally this 'primary phlogopite' usually seems to be a replacement phase rather than part of the original paragenesis (Green, H. W. II, and Boullier, A. M., not yet published), but its coarse grain size and apparent stability with respect to the other phases of the rocks strongly indicates an origin which predates incorporation into the kimberlite magma. Thus the rocks do contain small quantities of water.

We have not yet considered the chemical and textural differences present in the xenoliths. The coarse grained harzburgites correspond to region *a* (Fig. 2) and the highly deformed lherzolites exist in region *b* (Fig. 1). We expect such an abrupt textural and chemical transition because the undepleted diapir flows plastically on its margins as it rises through the previously depleted, annealed upper mantle. The top of the diapir would be expected to be the only place where undepleted mantle could be sampled because only this region has lost enough heat to miss the solidus and stay intact.

Macgregor³ has described a second group of xenoliths in the Kimberley region (Fig. 3). This atypical group plots at distinctly higher temperatures than the first, 'normal', group which is remarkably similar from pipe to pipe and from region to region. If there are two geotherms in the same region then there cannot be a steady-state situation. The second geotherm could represent the 'tail' of an earlier diapir which continued to rise at a reduced velocity after it had been partially melted. Alternatively, the second geotherm could represent an earlier diapir which rose more slowly and consequently never reached the melting point (for example, a velocity of ~0.1 cm yr⁻¹ would produce the observed slope with our model). In either case the first diapir must precede the second diapir, which brings the fragments to the surface, by only a few tens of millions of years otherwise the internal temperature of the first diapir would be reduced to the normal, unperturbed geotherm. An interesting difference between these two possible explanations is that for the former possibility the older diapir 'tail' could be mineralogically and texturally indistinguishable from normal lithosphere of region *a* (Fig. 2) but in the latter case the first diapir should be chemically undepleted even though it might be texturally similar to normal lithosphere. Unfortunately, the data³ are not separated in such a way as to allow these possibilities to be tested.

The discovery³ of the 'second geotherm' in the Kimberley region emphasises the need for a time-dependent explanation for the geochemical data, but it is not the only observation which supports this class of model. To make a steady state model fit the inflection in the palaeogeotherms a large and discontinuous change in thermal conductivity is required^{1,3}. Variation in the depth of the inflection point and its correlation with the xenolith textural types indicate that the effect would have to be produced by the preferred orientation in the deformed rocks or by some inherent effect of deformation on thermal conductivity. Because of the rather weak preferred orientations in these rocks (A. M. Boullier, personal communication) an anisotropy of *K* of more than a factor of 50 would be necessary. Similarly, any effect of crystal defects on the thermal conductivity would seem to be small because such an effect depends on high concentrations of defects¹⁷, whereas the high temperature deformation undergone

by these rocks produces recrystallised grains with extremely low defect densities (Green, H. W. II, and Boullier, A. M., not yet published).

In an earlier model^{1,5,8} shear heating in the low velocity zone is proposed to explain the steepened portion of the geotherms. Our calculations show that rates of strain exceeding 10⁻¹¹ s⁻¹ would be required to fit the data quantitatively, even assuming that there is no loss of heat by conduction. We consider such rates to be geologically unreasonable and therefore believe that the heat is better provided by a convective mechanism. Our diapiric model is the extreme case in which all of the heat is produced by convection. Other models can be constructed in which both upwelling and shear heating are involved¹⁸, but they are more complicated and therefore more difficult to apply quantitatively.

Two additional consequences of the diapiric model are of considerable interest for understanding upper mantle processes. First, if the model is to be valid, the HALVZ cannot be partially melted; either another fluid¹⁹ or a completely different mechanism²⁰ must be sought to explain the seismic data. (Interestingly, the same conclusion seems to be required for any model which equates the deformed xenoliths with the HALVZ because these rocks are unmodified and contain no distributed melt phase (Green, H. W., and Boullier, A. M., not yet published).) Second, if xenolith-bearing basaltic eruptions in tectonic and oceanic areas are also preceded by diapiric upwellings, as seems probable to us, then the flow mechanisms evident in the xenoliths reflect the mechanisms of diapirism which may not be the same as those of flow in the asthenosphere. Thus, the conclusions^{21,22} that flow in the upper mantle is dominated by movement of dislocations which are climb-controlled does not necessarily apply to asthenospheric flow. These arguments do not, however, contravene those based on post-glacial uplift data and power-law creep in experiments²³.

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Neural antigens of morphologically differentiated neuroblastoma cells

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Murine neuroblastoma cells in serum-free medium undergo correlative morphological and serological differentiation. The antigens specific to the differentiated cells are found in large quantities in mouse brain.

MURINE neuroblastoma cells can be induced to differentiate morphologically so as to resemble mature neurones if they are maintained in medium without serum¹. This morphological differentiation is often not accompanied by biochemical differentiation, as studies of neurotransmitter biosynthetic enzymes², glycosphingolipid composition³, tryptic glycopeptides⁴, mouse alloantigens⁵ and acrylamide gel patterns of solubilised membranes⁶ have shown. Similarly, studies of the development of the ability to generate an action potential and of sensitivity to electrical and chemical stimulation, have produced no consensus on any correlation with morphological differentiation⁷⁻¹⁰.

We have described a set of cell surface antigens on morphologically differentiated cells derived from clone N18 neuroblastoma¹¹. They are also present in large quantities in particulate preparations of mouse brain, but not in spleen, liver or kidney. Their appearance is the only reported specific change in the cell membranes that correlates with the morphological differentiation of cultured neuroblastoma cells.

We report here that: (1) serological differentiation of N18 neuroblastoma cells correlates with the extension of processes in serum-free medium and (2) direct binding experiments with ¹²⁵I-labelled immunoglobulin confirm and partially quantify the presence of neurone-specific antigens found only on morphologically differentiated neuroblastoma cells.

Antisera specific to differentiated clone N18 murine neuroblastoma cells were produced by immunisation and adsorption as before¹¹. The increased binding of antibodies (at saturating concentrations) to N18 cells maintained for 5 d in serum-free medium (N18-SF) compared with the binding to N18 cells grown in suspension culture on bacteriological plates⁶ (N18-sus) implies that new antigens were present on the morphologically differentiated N18-SF cells (Fig. 1). Binding of ¹²⁵I-immunoglobulin to morphologically differentiated neuroblastoma cells, undifferentiated cells and mouse tissue correlated well with their antigenic activities measured by indirect immunofluorescence and complement fixation¹¹.

To examine the relationship between process formation and the presence of new antigens on neuroblastoma cells in serum-free medium we used the C1300 neuroblastoma clone 10D (ref. 11), which does not extend processes in serum-free

medium. The serological reactivity of 10D cells grown with serum was indistinguishable from that of N18-sus cells measured by complement fixation, immunofluorescence or saturation binding (ref. 11 and Table 1). In addition, 10D cells maintained in serum-free medium for 5 d (10D-SF) neither extended processes nor bound more immunoglobulin than did N18-sus or 10D cells grown in complete medium (Table 1). The presence of new antigens on N18-SF cells was thus related to the formation of processes, and not to an effect of maintenance in serum-free medium.

Morphology and antigenicity studies

We next examined the serological reactivity and morphology of N18 cells maintained without serum for less than 5 d in an attempt to relate antigenicity and morphology. Morphological development increased steadily for 5 d. After 6 d the cultures tended to degenerate. Complement fixing ability (Fig. 2) of N18 cells in serum-free medium decreased initially for 2 d, probably because the cells were derived from near-confluent monolayers, and then increased for 3 d. Confluent cells were to some degree morphologically differentiated, thus a period in nonconfluent conditions may have been necessary for the antigens associated with this differentiation to be removed from the cell surface. Near-confluent cultures grown in complete medium had reactivity similar in both

Table 1 Antibody binding to neuroblastoma cells

Antigen	Immunoglobulin bound at saturation (ng)	% of N18-SF Value
N18-SF	48	100
N18-sus	37	78
10D	36	75
10D-SF	35	72

Values are the average from three experiments.

complement fixation and saturation binding assays to that of cells cultured for 3 d without serum. There was thus little serological differentiation during the first 2 d in serum-free medium, followed by a steady increase in antigenic reactivity.

In general the primary morphological change that correlates with increasing immunological reactivity is the formation of steadily lengthening processes on the cells. Morphological development seems to precede antigenic development. The multispecificity of our antiserum is undoubtedly an important source of difficulty in attempting to correlate

discrete antigenic and morphological events.

We next examined the relationship of the new antigens to the antigens of various mouse organs. Our antisera were produced to N18-SF cells, and so the reactive antigens present on the other cells and tissues tested should have been subsets of those found on N18-SF. No relationship between (for example) the antigens present on brain and liver can however, be inferred from Fig. 1. The liver anti-

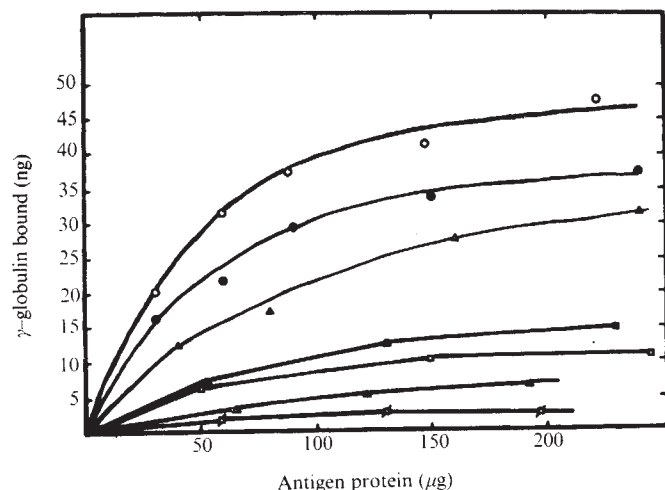


Fig. 1 Binding of ^{125}I -labelled immunoglobulin to mouse organs and cultured neuroblastoma cells. Antisera production and cell culture have been described. All experiments utilised the bleeding from rabbit 147 on the 20th day of the immunisation protocol¹¹. Unless otherwise noted, all operations were performed at 0° – 8°C . γ -Globulin was isolated by the method of Fahey and Horbett¹⁴ and labelled by a large scale modification of the method of Greenwood *et al.*¹⁵ using carrier-free ^{125}I (New England Nuclear Corp.). Labelled immunoglobulin was separated from unbound iodine on Sephadex G25, diluted with an equal volume of 5 mg ml^{-1} bovine albumin in isotris buffer¹⁶, and stored in aliquots at -20° until use. The specific activity was 0.7 Ci g^{-1} . Cells and organs were prepared as before¹¹. Brain samples did not include the olfactory bulb. Spleen samples included only the expressed cells and not the capsule. Whole liver and kidney were used. Heart samples were taken from the caudo-lateral left ventricle wall. There were no substantial differences in saturation binding with tissues from A/J, B₆AF/J, C3H, C57B, HeJ or Swiss Webster mice. Labelled immunoglobulin ($260\text{ }\mu\text{g}$) was shaken for 5 h in a mock adsorption procedure. After filtration, $5\text{ }\mu\text{g}$ was added to each antigen sample. All tissues are from a 60-d-old Swiss Webster mouse. The ^{125}I -immunoglobulin and antigen PM were combined in isotris buffer plus 10% normal rabbit serum (final volume of 1.5 ml) incubated 12–16 h, then centrifuged for 23 min at $45,000\text{g}$. The supernatant was discarded. The pellet was resuspended by vortexing for 15 s in 1.2 ml isotris containing 2 mg ml^{-1} bovine albumin (BA). After a second centrifugation for 26 min at $45,000\text{g}$, the pellet was resuspended in 1.2 ml isotris containing 2.0 mg ml^{-1} BA. The suspensions were then filtered (at room temperature) over a Whatman GF-A glass fibre paper which had been presoaked overnight in 2.5 mg ml^{-1} BA in isotris. The tube was washed twice with 2 ml isotris, and the washes filtered. The filters were rinsed twice with 2 ml absolute ethanol and dried for at least 30 min at 75°C before counting. For each curve the ng of antibody bound by the equivalent amount of HeLa cells is subtracted to obtain the specific binding. This background in the linear portion of the binding curve is 10–15% of the values obtained using N18-SF as the antigen. That the γ -globulin which sticks to HeLa cells is bound nonspecifically is demonstrated by the observations that (a) this binding is a linear nonsaturating function of added HeLa cell protein, (b) exhaustive adsorption of antiserum with an antigen reduces the binding of antiserum to that antigen down to but not below the levels obtained with HeLa cells, and (c) adding excess unlabelled antiserum reduces binding of labelled antiserum to N18-SF down to but not below the level obtained with the same amount of HeLa cell protein. HeLa cells do not react with this antiserum in either complement fixation or immunofluorescence assays. O, N18-SF; ●, N18-sus; △, brain; ■, kidney; □, liver; ▲, spleen; ▢, heart.

gens, in whole or in part, could be a subset of the brain antigens, or the two sets of antigens could be independent. To determine the relationship(s) between them, we performed binding experiments using ^{125}I -immunoglobulin adsorbed with particulate material (PM) from various mouse organs. Liver was chosen as a non-neural source of antigen because it binds relatively large amounts of the immunoglobulin (compared with other non-neural tissues) and has low levels of innervation.

Adsorption of immunoglobulin with particulate preparations from liver removed antibodies that bind not only to liver but also to all the other organs and cells tested (Table 2). Antibodies that bind to spleen and heart were removed when the immunoglobulin was adsorbed with liver PM. Therefore the reactive antigens found on these tissues were probably identical to those of liver. In a reciprocal experiment spleen adsorbed all binding activity to heart and liver (data not shown). A small amount of the antibodies that bind to kidney were not removed by adsorption with liver PM. It is not clear whether these antibodies reacted with the

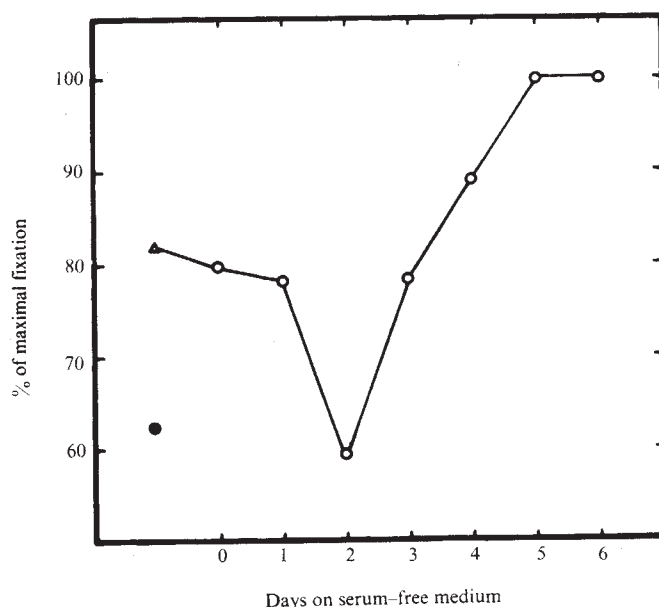


Fig. 2 Complement fixation of N18 cells maintained for varying periods on serum-free medium. Data are expressed as percentages of the maximal fixation obtained with N18 cells maintained 5 d on serum-free medium. Confluent or late log phase cultures were treated with Viokase and plated as before¹¹ to obtain cultures subsequently maintained in serum-free medium. △, Late log or confluent cultures; ●, N18-sus; O, N18.

neural antigens which were present as a consequence of the innervation of the kidney, or with some other antigen not shared by the liver. Binding of liver-PM-adsorbed immunoglobulin to brain, N18-sus and N18-SF cells was reduced by approximately equal amounts. The antigens found on liver seemed to be present on all the cells and organs tested and are probably common to all mouse cells. Immunisation of a xenogeneic animal, the rabbit, would be expected to raise antibodies to species-specific antigens.

As adsorption of the immunoglobulin with brain PM removed all antibodies which bind to other organs (Table 2), brain must contain all the antigens found on the other organs, but not all those present on the cultured neuroblastoma cells. The absolute binding measured in ng to N18-SF cells of brain-adsorbed antibody was reduced more than the binding of such antibody to N18-sus. This reduction suggests the presence of neural antigens found on N18-SF but not on N18-sus. The difference in binding between N18-SF and N18-sus [$\Delta(\text{SF-sus})$] could be reduced 67% by ad-

Table 2 Organ adsorption of antibody binding activity

Antigen used for test	% Antibody binding activity adsorbed by:	
	Brain	Liver
N18-SF (differentiated)	75 (32.0)	38 (16.0)
N18-sus	79 (27.2)	51 (17.5)
Brain	88 (22.0)	56 (15.0)
Kidney	95 (14.0)	75 (10.5)
Liver	93 (10.0)	>95 (10.8)
Spleen	>95 (6.2)	92 (5.8)
Heart	>95 (2.5)	>95 (2.5)
Δ (SF-sus)	67 (6.8)	< 5 (0.1)

Two aliquots of 130 μ g of 125 I-immunoglobulin were adsorbed with 5 mg of liver and brain respectively. This is the amount of antigen necessary to adsorb 90% of the antibodies which bind to brain and about 1.5 times the amount necessary to adsorb 90% of the antibodies which bind to liver. A volume containing 5 μ g of adsorbed antibody was added to each antigen sample (150 μ g). The mixtures were incubated for 12 h at 8°C. All cells and organs were the same preparations as those described in Fig. 1. Numbers in brackets are ng of antibody binding activity removed.

sorption with brain PM but less than 5% by adsorption with liver PM (Table 2, last line). N18-SF is the only antigen source able to adsorb totally the binding immunoglobulins in the antiserum.

These and other adsorption experiments established the following relationships among the sets of antigens reactive with this antiserum. (1) The set of antigens present on a tissue includes as a subset all reactive antigens of tissues with a lesser total reactivity. For example, the set of reactive antigens present on liver PM includes as subsets those reactive antigens present on spleen and heart PM, while the set of reactive antigens present on brain includes all those of kidney, liver, spleen and heart. (2) Approximately two-thirds of the antigens present only on the morphologically differentiated form of N18-cells are found only on neural tissue. Among antibodies that bind to brain are some that bind to morphologically differentiated but not

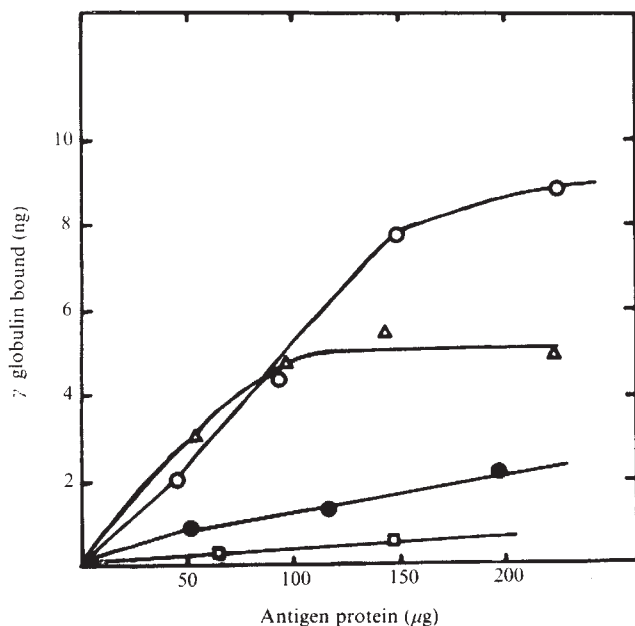


Fig. 3 Binding of 125 I-immunoglobulin adsorbed with N18-sus. 125 I-immunoglobulin (120 μ g) was adsorbed with 3.3 mg of N18-sus PM for 16 h. A volume containing 5 μ g was added to each antigen sample and the mixture was incubated 14 h at 8°C. Organs were from a 45-d-old C57BL mouse. Symbols as in Fig. 1.

to undifferentiated neuroblastoma cells.

We attempted to confirm the neuronal specificity of Δ (SF-sus) by further methods. Adsorption of the immunoglobulin with N18-sus removes all antibodies that bind to all cells and tissues tested, except N18-SF and brain (Fig. 3). For such an adsorbed immunoglobulin preparation, Δ (SF-sus) was 6.0 ng, while Δ (brain-sus) was 3.4 ng or 57% of Δ (SF-sus). This value is close to the 67% of Δ (SF-sus) already given (Table 2) as specific to neural tissue. Each of these calculations gives a minimal value because in each

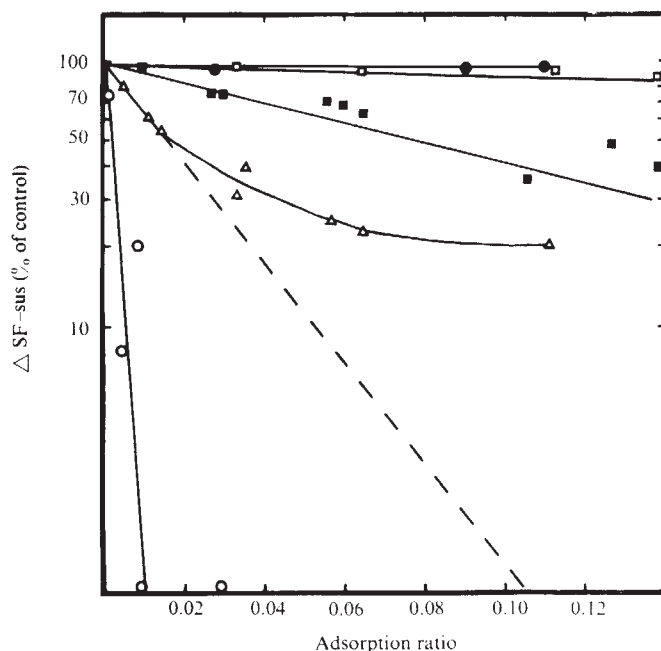


Fig. 4 Exhaustive adsorptions with PM from mouse organs. The reduction in Δ (SF-sus) is plotted as a function of the amount of PM used to adsorb. Spleen cells could not be used in these exhaustive adsorption experiments, due to their affinity for rabbit γ -globulin. This binding is probably due to the presence of receptors for the F_c portion of immunoglobulin molecules¹². The adsorption ratio is defined as the mg of organ PM per μ g 125 I-immunoglobulin used in the overnight adsorption. By comparison this ratio in the adsorptions of Table 2 is 0.038. The adsorption ratios at which 90% of the binding activity to each antigen is removed by adsorption with that same antigen are liver, 0.026; brain, 0.038; N18-SF, 0.018. Values for muscle and kidney were not determined. The lines drawn are linear regression fits to the data points in all cases except brain. The dashed line is an extension of the line of best fit to the first three brain adsorption points. The remainder of the solid curve for brain is a free-hand fit of the points. O, N18-SF; Δ , brain; $\blacksquare$, kidney; $\square$, liver; $\bullet$, skeletal muscle.

case the adsorption of antibody molecules was incomplete. Any unadsorbed binding material was assumed to not be specific to neural tissue.

If adsorption and subsequent direct binding assays were performed with particulate preparations from monolayer 10D-neuroblastoma cells rather than N18-sus cells, the ability of brain to adsorb the remaining (N18-SF specific) immunoglobulin preferentially was unchanged (data not shown). Thus, brain reacted preferentially with an antiserum made specific for the morphologically differentiated state of neuroblastoma cells, whether N18-sus or 10D monolayer PM was used for adsorption. In summary, antigens found only on the differentiated form of N18 neuroblastoma cells were also present on brain but not other organs, when assayed by both direct binding and adsorption.

To determine whether antigens present only on the

morphologically differentiated form of N18 neuroblastoma cells are present in lesser concentrations on particulate material from organs other than brain, we performed binding studies with immunoglobulin exhaustively adsorbed with skeletal muscle, liver and kidney as well as brain PM. The adsorbed immunoglobulins were tested against both N18-SF and N18-sus to determine whether antibodies reactive with antigens unique to the differentiated neuroblastoma cells had been removed.

Liver and skeletal muscle PM did not adsorb significant amounts of the Δ (SF-sus)-specific immunoglobulins (Fig. 4). Adsorptions with brain preparations produced a multiphasic curve. The ratio of the slope of the adsorption curve with N18-SF to that of the initial slope of the brain adsorption curve (dashed line) is 12 : 1, indicating that the specific activity of Δ (SF-sus) antigens was twelve times greater on N18-SF cells than on brain. The slope of the brain adsorption curve seems to be reduced at about 50% reduction of Δ (SF-sus). Therefore the remaining Δ (SF-sus) antigens adsorbed by brain were present in brain with specific activities less than one-twelfth of the activity of these antigens in N18-SF cells. In total 80% of the Δ (SF-sus) antigens was adsorbed by brain PM in exhaustive procedures.

If the linear regression line is extrapolated, the specific activity of the Δ (SF-sus) antigens in kidney is one-seventh that of brain (1/84th the specific activity of these antigens in N18-SF). However, the kidney adsorption curve appears to saturate at ratios of 0.10 to 0.14. (Larger adsorption ratios were difficult to examine because of nonspecific effects of large amounts of PM.) Tissues other than brain might be expected to contain small amounts of neural antigens due to their intrinsic innervation. If the kidney

the antiserum by adsorption with PM from either brain, liver or spleen.

Antibodies to the second class of antigens present on morphologically undifferentiated cells can be removed only by adsorption with particulate preparations from brain, and not by any other organs tested. By this criterion and also by direct binding studies, these are neural antigens (type II in Table 3).

Antibodies to the third class of antigens present on morphologically undifferentiated N18 cells are not adsorbed by any of the organs tested. These constitute type III and may be the result of cellular adaption to culture. They may be present only on the tissue (sympathetic nervous system) or cell of origin of the tumour. They might even be related to the malignant transformation of these cells and (or) to their associated A type virus particles¹³. These antigens are called cell-specific in Table 3.

Adsorption experiments demonstrated that morphologically differentiated N18 cells possess all these classes of antigens. In addition, such cells also have antigens related specifically to their morphological differentiation. Approximately half to two-thirds of the antibodies unique to morphologically differentiated cells are adsorbed only by PM from brain (type IV in Table 3). The remainder of the antibodies to antigens unique to morphologically differentiated cells are similar to the third class of antigens described here in that they are not adsorbed by any mouse organ or cell (including 10D-SF) except N18-SF (type V). We hope subcellular fractionation techniques and (or) tolerance inducing methods will facilitate the production of more specific antisera that may be used for detailed study of individual antigenic correlates of morphological differentiation of the murine neuroblastoma in culture.

We thank B. Konya, S. Redmond and P. Tyson for technical assistance, and Dr John Fahey for critical review. This work was supported in part by a contract between the US Atomic Energy Commission and the University of California. R.A. was supported in part by a National Institute of Mental Health training grant.

Table 3 Antigen classes of murine neuroblastoma cells

Proposed antigen class on:	Presence of antigen on:				
	HeLa	Liver Spleen and so on	Brain	N18- sus	N18- SF
Undifferentiated cells					
I Common Mouse	—	+	+	+	+
II Neural	—	—	+	+	+
III Cell-Specific	—	—	—	+	+
Differentiated cells only					
IV Neural-specific	—	—	+	—	+
V Cell-specific	—	—	—	—	+

—, Will not adsorb; +, will adsorb.

adsorption curve does saturate at 50%, then (i) approximately 30% of the Δ (SF-sus) antigens cannot be detected by this exhaustive adsorption method in any organ but brain; (ii) half the Δ (SF-sus) antigens are enriched in brain by a factor of seven to one hundred or more when compared with other organs, and (iii) the remaining 20% cannot be detected in any of the organs tested, including brain. Similar calculations, based on complement fixation¹¹ and saturation binding analysis, suggest that the set of antigens unique to morphologically differentiated N18 cells is present in at least 100 times the specific activity on such cells as on morphologically undifferentiated neuroblastoma cells (N18-sus, 10D or 10D-SF).

Proposed antigen classes

The xenogeneic antiserum we used revealed a spectrum of particulate antigens on N18 neuroblastoma cells. Morphologically undifferentiated cells have antigens which seem to be shared by all mouse organs and histocompatibility types. They are the mouse common antigens (type I in Table 3). Antibodies to these antigens can be removed from

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Age of early Acheulian industries from the Peninj Group, Tanzania

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Geophysical data strongly indicate that stone assemblages, which include bifaces and cleavers, from East Africa, are at least a million years old. Since these artefacts seem to be much older than any of their known counterparts in Europe, the question of the date of man's expansion from the tropics into cool, temperate regions is raised for discussion.

ONE of the most long lasting and widespread classificatory entities in human prehistory is the set of stone industries collectively known as Acheulian¹⁻³. These industries are characterised, among other things, by the prominence of a series of tools variously called 'bifaces' or 'hand axes'. Representative assemblages are widely distributed; they occur in most regions of Africa, in southern and atlantic Europe, in the Levant and across into peninsular India. Isolated finds referable to the Acheulian have been reported from northern and central Europe, and from continental Eurasia, but north-east of the Alps, Caucasus, Zagros and Himalayan chains of mountains this kind of industry is conspicuously absent or at best rare and atypical.

With regard to age, it has long been known that the Acheulian industrial pattern was in practice through much of that rather vague segment of geological time classified as the 'Middle Pleistocene', though some of the last Acheulian assemblages to be made, belong in the early part of the 'Upper Pleistocene'. The upper and lower boundaries of the 'Middle Pleistocene' are not well fixed stratigraphically and evaluations of their age in years have been highly uncertain and variable from region to region. Consequently estimates of the time span represented by the Acheulian have also been varied and uncertain. In particular the relative age of early representatives of the industry from different regions has been in doubt.

We are reporting here new geophysical evidence from East Africa which provides strong indications of the chronometric age of early Acheulian assemblages in that subcontinent. The evidence raises very important questions with regard to inter-regional correlations and with regard to the timetable for changing patterns in the biogeography of early man. Although we raise these issues, their resolution must lie in part in the application of geophysical techniques to artefact-bearing sections other than those in East Africa.

The new data come primarily from a group of sedimentary and volcanic formations in Northern Tanzania, namely that of the Peninj, west of Lake Natron. The evidence consists of potassium-argon dates and palaeomagnetic determinations based on samples interstratified with artefact-bearing horizons. Although we are continuing to elaborate and check the evidence, the main features of the results seem sufficiently well established to warrant summary publication.

The Peninj Group

In an area about 50 miles north east of Olduvai, on the west side of Lake Natron a long sequence of sediments and volcanics has yielded a hominid fossil, mammal fossils, early Acheulian assemblages and geophysical evidence for dating. The regional

sequence has previously been described^{4,5} but is summarised here in Fig. 1 and Table 1. A series of K-Ar age determinations has been made by one of us (G.C.) and is also shown in Table 1.

Assemblages of early Acheulian artefacts were obtained by excavation from two localities in the northern sector of the 40 km strip over which the sedimentary sequence has been studied. The archaeological horizons were stratified within the Humbu Formation and in each case the artefacts closely overlie a major basaltic tuff unit.

One of the units dated by K-Ar, the 'Wa-Mbugu basalt' is an olivine basalt flow bedded within basaltic tuffs of the Humbu Formation some 15-20 km to the south of the sites. Another dated flow, the 'intra Moinik basalt', is stratified within the base of the overlying Moinik Formation in outcrops about 8 km to the north. Unless the stratigraphic boundary between the Humbu Formation and the Moinik Formation is markedly time-transgressive from north to south, these two units bracket the age of the Acheulian occurrences. Field relations and the nature of the contrast between the two units make time transgression seem unlikely. In any event, the two flows clearly are close in age to the age of the Acheulian industry. An australopithecine mandible was recovered from beneath the basaltic tuffs and is definitely older than both flows^{6,7}.

Potassium-argon age determinations

Table 1 shows the results of K-Ar and palaeomagnetic determinations made on various volcanic rocks from the West Natron succession. These K-Ar measurements were made by the standard techniques in use in the Berkeley K-Ar laboratory⁸. The palaeomagnetic results were obtained by T. Reilly working in Nairobi on samples collected by Isaac and Musset⁹. Although there is a fair degree of concordance between stratigraphy and K-Ar ages, the results do not show complete internal consistency

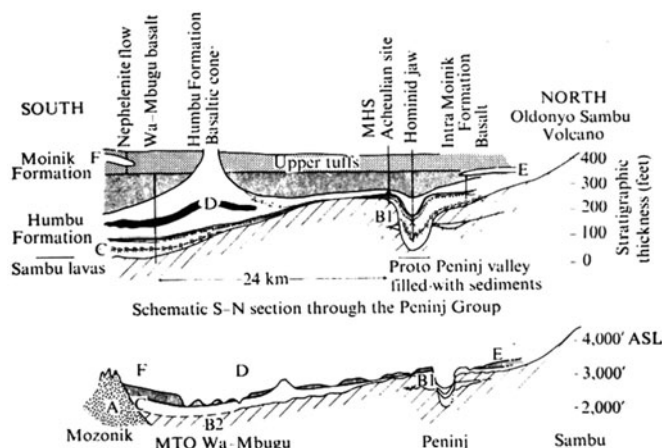


Fig. 1 Diagrammatic north-south sections through the Peninj Group in the West Natron area. The upper section shows the disposition of stratigraphic units without regard to topography. The lower section indicates topographic relations. Letters refer to lava flows listed in Table 1. (For a map see ref. 4, Fig. 1.)

Table 1 Potassium-argon and magnetic polarity determinations made on the succession of volcanic rocks in the West Natron area

Stratigraphic unit and rock type	Run number	K (wt %)	Radiogenic Ar ¹	Radiogenic Ar (%)	Apparent age $\times 10^6$ (Myr)	Polarity	Degree of alteration
Moinik Formation							
(F) Nephelinite	KA 1814f	Grossly inconsistent with stratigraphy				R	
intra Moinik basalt	KA 2410	1.02	0.210	14.4	1.33 ± 0.05	?	5% zeolite
(E)	KA 2589	1.01	0.249	10.6	1.38 ± 0.09	?	
Humbu Formation							
(D) Wa-Mbugu olivine basalt (1964 sample)	KA 1754	0.729	0.200	26.0	1.55 ± 0.03	N	3–5% analcime
	KA 1754R	0.729	0.294	10.7	2.27 ± 0.06	N	
Wa-Mbugu olivine basalt (1969 samples)	KA 2382	0.812	0.181	5.7	0.96 ± 0.10	N	10–15% zeolite
	KA 2382R	0.678	0.220	2.1	1.21 ± 0.36	N	10–15% zeolite
	KA 2578	0.765	0.132	2.7	0.97 ± 0.25	N	10–15% zeolite
	KA 2646	0.750	0.161	6.9	1.21 ± 0.10	N	10–15% zeolite
(C) Hajaro basalt	Too altered for dating purposes					R	
Sambu lavas							
(B ₁) Basalt from Peninj Gorge	KA 1755	0.949	0.321	15.4	1.90 ± 0.07	N	~2% zeolite
	KA 1755R	0.949	0.298	18.6	1.77 ± 0.04	N	
(B ₂) Basalt from Malambo Gorge	KA 2570	0.579	0.361	4.9	3.50 ± 0.46	R	
Mozonik Volcanics							
(A) Biotite lava	KA 1757	6.86	3.88	18.4	3.18 ± 0.08	?R	
Results grossly inconsistent with stratigraphy:							
(F) Moinik Formation nephelinite	KA 1814	5.84	4.82	40.3	4.60 ± 0.07	R	
	KA 2250	6.27	2.99	49.4	2.68 ± 0.06	R	

and some interpretation is necessary. Palaeomagnetic data for several of the lava flows provide valuable control in assessing the reliability of the apparent ages.

R. L. Hay has carried out petrographic and X-ray diffraction examinations of several of the samples in order to assess the degree of alteration. Of the samples used in runs KA 2382, 2578 and 2646 in a letter, he reports as follows: "A little of the zeolite is isotropic (= analcime?) but most is weakly birefringent. A diffractogram indicates a substantial amount of phillipsite which is presumably the birefringent zeolite". Hay's estimates of the overall percentage of zeolites are shown in Table 1. There are indications that the most heavily zeolitised lavas may be giving anomalously young ages.

Interpretation of the individual measurements must now be discussed. The oldest unit dated is the Mozonik Volcanics for which biotite flakes from tuffs give an age of 3.2 Myr (KA 1757). A lava flow elsewhere on Mount Mozonik showed reversed polarity and presumably part of the volcano's active life belonged in the Gilbert Reversed Epoch.

The Sambu lavas consist of a thickness of more than 1,000 feet of basalt flows apparently originating from Oldoniyo Sambu (Fig. 1). These lapped around the Mozonik volcanic centre. The flows have normal polarity except for thin localised flows at the very top of the succession. During an interval between a lower set and an upper set of flows, a major canyon was cut and filled with sediments. Under chronological scheme A in Fig. 2 the dates of 3.5 ± 0.46 Myr would be accepted and the dates of 1.9 and 1.77 Myr (KA 1755 and 1755R) for one flow in the upper set would be regarded as erroneous and the whole sequence assigned to the Gauss Normal Epoch. Under scheme B, the younger K-Ar results would be accepted as being consistent with an Olduvai event age. The first interpretation seems to be in better accord with the large scale of the formation.

The Wa-Mbugu basalt is bedded within the Humbu Formation at a level correlated with basaltic tuffs that overlie the australopithecine mandible and underlie the Acheulian industry. This single localised flow consistently showed normal polarity. A scatter of K-Ar apparent age values has been obtained, which seem to fall into two groups. A sample which showed only relatively slight alteration has given ages with a mean of 1.91 Myr (1754 and 1754R) whereas two samples with 10–15% of zeolite yield apparent ages between 0.96 and 1.21 Myr (2382, 2382R, 2578 and 2646). If the older dates are accepted an Olduvai Event age is implied, if the younger ones are accepted—a Jaramillo Event age.

Two runs on a sample of the intra Moinik Formation basalt flow have produced consistent results with a mean of 1.35 Myr (2410, 2589). Unfortunately the polarity of this unit is unknown since all samples collected turned out to have been struck by lightning.

In the southernmost exposures a nephelinite flow is stratified within the upper tuff complex of the Moinik Formation, well above the equivalent level of the artefacts. This flow has reversed polarity (Site MZN113 of Reilley⁹) but attempts at K-Ar dating have produced anomalous results—2.6 and 4.6×10^6 Myr. Nephelinites, in our experience and in that of other laboratories, frequently give unreliable results and attempts to date this rock have been abandoned. The polarity evidence is, however, of great importance in evaluating the other determinations. Since the nephelinite with reversed polarity is stratified above the Wa-Mbuga Basalt in the same sections, the normal polarity of the basalt cannot pertain to the Brunhes Normal Epoch but must belong to the Jaramillo or Olduvai Events, as implied also by the K-Ar results.

Figure 2 shows alternative chronologies for the Peninj Group. Alternative A involves acceptance of the date of 1.35 Myr for the intra Moinik basalt and the older set of ages for the Wa-Mbugu Basalt. Under this scheme the 1.77–1.9 Myr age for a Sambu lava flow must be rejected as stratigraphically inconsistent. Alternative B depends on rejection of the intra Moinik basalt date of 1.35 Myr and acceptance of the younger scatter of dates for the zeolitised Wa-Mbuga Basalt samples. We regard alternative A as more likely to prove correct.

While this paper was being revised, McIntyre, Mitchell and Dawson¹⁰ reported K-Ar dates for volcanics from the area immediately to the south of the Natron basin. They conclude that major movements occurred along the Manyara-Natron

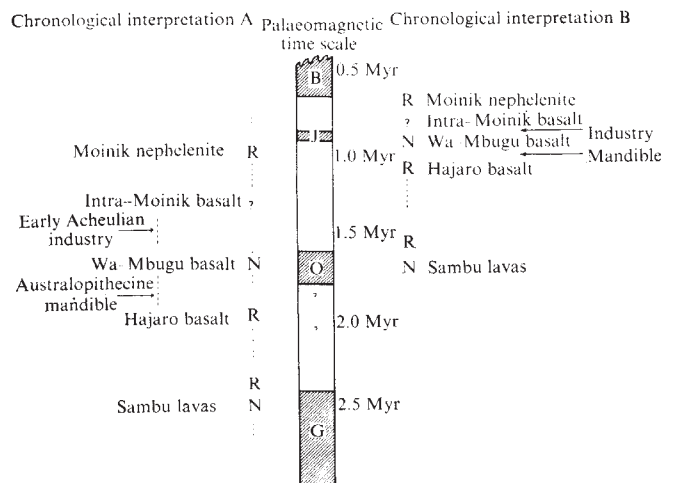


Fig. 2 The West Natron succession of lavas shown in relation to two alternative patterns of correlation of the available potassium-argon and palaeo-polarity data. Alternative A is favoured—see text.

Table 2 K-Ar or palaeomagnetic age determinations for Acheulian sites

Industry	Site	Apparent age $\times 10^6$ (Myr)	Comments and Source
Late Acheulian	Kapthurin	< 0.02	Ref. 24
Acheulian	Torre in Pietra	≤ 0.429	Mean of 7, ref. 8
Acheulian	Ologesailie	0.425	Ref. 25
Lower Acheulian and Developed Oldowan	'Ubeidiya	≥ 0.64	Ref. 21
		≥ 0.68	
Acheulian	Kariandusi	0.928	Ref. 9
		0.946	
		1.1	
Lower Acheulian	Olduvai EFHR	~ 1.4	(palaeomagnetic) Ref. 16

This list is restricted to instances where the sample source is closely associated with the formation in which the sites are stratified. Several of these dates are problematic. For a general review see ref. 23.

fault between 1.15 and 1.2 Myr ago. This episode almost certainly corresponds to the fault dislocations which terminated the accumulation of the Humbu and Moinik Formations along the western flanks of the Natron basin. The new information lends strong support to chronological interpretation A.

Taken as a whole the geophysical data seem to be unambiguous in their indication of an age of the order of 1.0 to 1.5 Myr for the early Acheulian sites in the Humbu Formation. Table 2 lists other Acheulian occurrences, dated by K-Ar or polarity stratigraphy. The extreme paucity of data is apparent and it is for this reason that we have reported our results in spite of their lack of precision.

In addition to the K-Ar age determination there are a number of uranium decay series measurements on carbonates associated with Acheulian industries, for example those of the Riviera and the Casablanca region¹¹, the Afar¹² and Isimila in Tanzania¹³. Where these methods have given rise to finite age estimates they are less than 300,000 yr. In most instances the associated industry is a 'Middle' or 'Late' Acheulian, so that the uranium series dates do not at present contribute to the problem of estimating the age of the early Acheulian. Some geochemists have important reservations about regarding Th-U measurements on many materials, as 'dates'¹⁴.

Implications

Whichever alternative chronology is adopted from Fig. 2, the age estimates for the Humbu Formation industries are very much greater than the values commonly entertained for the earliest Acheulian in the Mediterranean basin or Europe where estimates based on stratigraphic considerations commonly range between 300,000 and 500,000 yr. And yet the combined evidence of the various East African sections gives a very strong indication that at least some stone tool kits of Acheulian type began to be made in East Africa more than a million years ago. The Olduvai evidence reported by M. D. Leakey¹⁵ and by Brock, Hay and Brown¹⁶ is unambiguous in its demonstration that the earliest Acheulian assemblages are of Matuyama Reversed Epoch age (that is ≥ 0.7 Myr), and the K-Ar dates and stratigraphy of the Olduvai sections like the Natron section implies an age of between 1.0 and 1.6 Myr.

Until recently it seemed possible that the apparent discrepancy between East Africa and Europe was caused primarily by a tendency of European stratigraphers to undervalue the duration of the 'Middle Pleistocene', but recent palaeomagnetic work has resulted in the determination of the position of the Brunhes-Matuyama boundary in two important European Pleistocene sections—namely, those of the Netherlands and of Poland. Expressed in biostratigraphic and climate-stratigraphic terms, the boundary has been found by Mont-Frans to lie in the lower portion of strata classified as 'Cromerian' in the Netherlands¹⁷ and Kukla has located it in the early Biharian of Poland¹⁸. The oldest stone tool assemblages which contain hand axes in Europe, however, are normally assigned to the 'Elster' (or Mindel) stratigraphic complex which succeeds the 'Cromerian'.

Evidence for hominid occupancy of Europe before Elster-Mindel times is very scanty with the allegedly late Villafranchian site of the Grotte du Vallonet near Nice constituting one of a very few important possible documents¹⁹. Another possible

pre-Mindel occurrence is that of Abbeville, where hand axes were collected by D'Ault du Mesnil in 1875–96 (ref. 20). These may have been of late Cromerian age, but full stratigraphic information is not now available.

The 'Ubeidiya Formation contains what seem to be the oldest geophysically-dated artefacts in Eurasia. Lava flows believed to be stratigraphically above Acheulian and Developed Oldowan occurrences, have been dated at 0.64 and 0.68 Myr (ref. 21).

Palaeomagnetic stratigraphy has not yet been reported for such important Mediterranean basin sequences as those of Latamne (Syria), Ternifine (Algeria) or Sidi Abder Rahman (Morocco). The Indian sites are similarly unknown geochronological quantities, though new work in Java may soon throw light on the age of the apparently 'non-Acheulian' *Homo erectus* population there²².

If, as seems likely, further work substantiates the present indications of an antiquity of a million years or more for the earliest East African Acheulian assemblages, and, if the apparent ages of less than half a million years are sustained for Europe, then understanding of the timing of developments in human cultural geography will have to be profoundly modified. It will be necessary to consider the possibility that hominids were essentially restricted to tropical and warm temperate zones for a much larger fraction of their evolutionary history than has hitherto been envisaged. For these questions to be resolved it is urgent that there be an intensification of efforts to secure chronometric determinations and palaeomagnetic data from Middle Pleistocene, artefact-bearing sedimentary sequences in areas outside East Africa.

The research at Peninj was a joint undertaking with Richard E. Leakey. The Tanzanian government kindly gave its permission and encouragement. Financial support came initially from the National Geographic Society and latterly as part of a National Science Foundation Grant. The final stages of the work was done during tenure by one of us (G.L.I.) of a Miller Professorship in the University of California. Dr Mary Leakey read a draft report and kindly gave advice.

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Mechanism of action of the toxic lectins abrin and ricin

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The mechanism of action of the plant toxins abrin and ricin is described. The toxins inhibit protein synthesis probably by inactivating the 60S ribosomal subunits, interfering with chain elongation. Only eukaryotic ribosomes are attacked by the toxins.

PROTEINS toxic to animal cells are widely distributed but in only a few cases is their mechanism of action at the cellular and molecular level known in detail. Recently, considerable progress has been made in work on the toxic proteins abrin and ricin, whose mechanism of action is now rather well understood. The facts learned about the binding of abrin and ricin to cell receptors and their subsequent uptake into cells is of considerable general interest, since recent evidence suggests that similar mechanisms may apply in the case of other toxic proteins and possibly also in the case of some protein hormones and growth factors.

Abrin and ricin, which are present in the seeds of *Abrus precatorius* L. (Leguminosae) and *Ricinus communis* L. (Euphorbeaceae) respectively, have been known since the end of the last century (for review see ref. 1). Previously it was thought that the toxic action of these proteins in animals is due to the strong haemagglutinating properties of abrin and ricin preparations. More recent work has shown, however, that the agglutinating activity is associated with closely related, nontoxic agglutinins present in the same seeds²⁻⁷ and that the highly purified toxins possess little or no agglutinating ability. Abrin and ricin have been reported to be more toxic to malignant than to normal cells and attempts to treat cancer in animals and man with abrin and ricin have been reported⁸.

Structure

Abrin and ricin are glycoproteins with molecular weights of 60,000 to 65,000 (refs 2, 4, 7, 9-11) (Fig. 1). The whole toxins move as single bands in polyacrylamide gels in the presence of sodium dodecyl sulphate, but on treatment with 2-mercaptoethanol, they are split into two peptide chains^{5,6,9-11}, moving at rates corresponding to molecular weights between 30,000 and 35,000 (Table 1). Thus, the toxins consist of an A and B chain, joined by SS bonds (Fig. 1). We have separated the two peptide chains in their native form by ion exchange chromatography^{5,6}. The isolated peptides thus obtained are not contaminated to any significant extent with the other chain. If a mixture of the isolated A and B chains is dialysed to remove 2-mercaptoethanol, the disulphide bridges between the two chains are reformed (S.O., A. M. Pappenheimer, and A. A. Harper, in preparation). The reconstituted proteins are almost as toxic as the native proteins (Table 2), demonstrating that the

peptide chains do not lose their activity during the separation. Hybrid proteins consisting of the A chain of abrin and the B chain of ricin and vice versa, were also prepared (S. O., A. M. Pappenheimer, and A. A. Harper, in preparation). Such hybrids have toxicities close to those of the native toxins (Table 2).

Both toxins consist of a neutral and an acidic peptide chain (Table 1). In the case of abrin, the A chain is acidic, whereas in ricin the B chain has the low pI value. In both toxins the

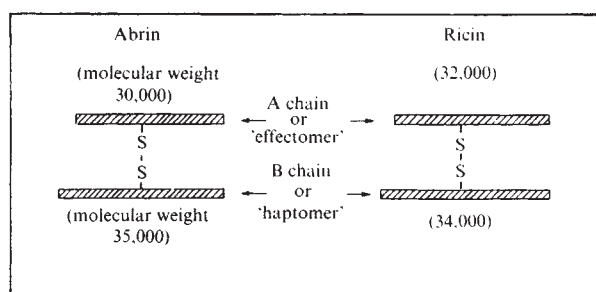


Fig. 1 Schematic structure of abrin and ricin.

B chains contain most of the carbohydrates (Table 1).

The structure and properties of abrin and ricin are surprisingly similar although they come from the seeds of two unrelated plants. Although the toxins are immunologically different (refs 5, 6 and 12 and S. O., K. R., A. P., and T. Christensen, in preparation) and show differences in their amino-acid composition (in preparation) the many similarities suggest that the toxins descend from a common ancestor molecule.

Effect in vivo

Abrin and ricin are extremely toxic to animals and man. After intraperitoneal administration the LD₅₀ doses in mice were 40 ng and 100 ng for pure abrin and ricin, respectively^{5,6}. These very small doses lead to death in the course of a few days, whereas larger doses have a more rapid effect. But animals never die earlier than 8-10 h after intraperitoneal administration of toxin. In animals intoxicated with large doses, haemorrhages in the viscera and serous cavities are found, whereas in animals treated with smaller doses multifocal necrosis develops in the parenchymous organs and in the germinal centres of the lymphatic organs¹³. Previous work revealed little or no change in oxygen consumption and energy metabolism, and the serum concentration of various metabolites studied remained unchanged in the early stage of intoxication¹⁴.

Table 1 Physical properties of abrin, ricin and their constituent peptide chains

Toxin	Molecular weight*	pI†	Carbohydrate content‡
Abrin	65,000	6.1	—
Abrin A chain	30,000	4.6	0
Abrin B chain	35,000	7.2	7.4
Ricin	65,000	7.1	—
Ricin A chain	32,000	7.5	2.4
Ricin B chain	34,000	4.8	6.5

*Measured by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate⁹.

†Measured by isoelectrofocusing (S. O., K. R., A. P., and T. Christensen, in preparation).

‡In % (w/w). Measured by gas chromatography (S. O., K. R., A. P., and T. Christensen, in preparation).

The selective inhibition of protein synthesis by abrin and ricin was first shown by Lin *et al.*^{14,15} and our data in Fig. 2 confirm their experiments. It is shown that when 1-ml samples of Ehrlich ascites cells were treated with 0.1 µg of the toxins, the incorporation of ¹⁴C-leucine into acid-precipitable material decreased after a lag time of about 30 min and stopped after 2 h (Fig. 2a). The incorporation of labelled thymidine was also inhibited by this concentration of the toxins (Fig. 2b), although after a longer lag time. On the other hand, the incorporation of ¹⁴C-uridine was only slightly inhibited by 0.1 µg ml⁻¹ of the toxins (Fig. 2c).

Different functions of the two peptide chains

The toxic effect *in vivo* seems to depend on the intact structure of the toxins¹⁶. Thus, we found that the isolated peptide chains had only ~1% of the inhibiting effect on protein synthesis in cells compared with that of the intact toxins (data not shown). A similar relationship was found when the toxic activity was tested in living animals (Table 2). A mixture of the two chains (reduced toxins) had the same low effect as isolated A or B chains. As pointed out above, reconstituted toxins or hybrid proteins show about the same toxicity as native abrin and ricin. (S. O., A. M. Pappenheimer, and A. Harper, in preparation).

Table 2 Biological properties of abrin, ricin and their constituent peptide chains

	Toxicity	Inhibition of cell-free protein synthesis*	Indirect haemagglutination†
	LD ₅₀ (µg)‡	Amount giving 90% inhibition (i µg ml ⁻¹)§	Amount giving visible agglutination (i µg ml ⁻¹)
Abrin	0.04	1.00	0.05
Abrin A chain	> 10.00	0.01	1.00
Abrin B chain	> 10.00	5.00	0.05
Abrin (reduced)¶	> 10.00	0.02	—
Abrin (reconstituted)	0.15	—	—
Ricin	0.10	1.00	0.04
Ricin A chain	> 10.00	0.01	5.00
Ricin B chain	> 10.00	3.00	0.04
Ricin (reduced)¶	5.00	0.03	—
Ricin (reconstituted)	0.20	—	—
Hybrid (abrin A chain/ricin B chain)	0.15	—	—
Hybrid (ricin A chain/abrin B chain)	0.30	—	—

* The cell-free system (0.5 ml) was an unfractionated rabbit reticulocyte lysate to which was added salt solution, an energy donating system, ¹⁴C-leucine and the 19 other (unlabelled) amino acids. The system is essentially that of Lingrel as described elsewhere²¹.

† Washed human erythrocytes (blood group B⁻) were mixed with toxins or their peptide chains in 1 ml of 10 mM Tris-HCl (pH 7.5), 0.14 M NaCl for 60 min at 20° C. Then 5 µl of antiabrin or antiricin was added and the samples were observed under the microscope after 9 h. Conditions as described elsewhere^{5,6}.

‡ The amount giving 50% death when injected intraperitoneally into mice as described elsewhere^{5,6}.

§ Measured after 60 min incubation at 30° C.

¶ Pretreated with 1% 2-mercaptoethanol overnight at 4° C.

|| Equimolar amounts of A chain and B chain were mixed and dialysed to remove 2-mercaptoethanol and reoxidise the disulphide bridge as described elsewhere (S. O., A. M. Pappenheimer, and A. Harper, in preparation).

The results indicate that only molecular species consisting of A and B chains joined by disulphide bridges have toxic effects on intact cells and living animals.

When the effect on protein synthesis of abrin, ricin and their constituent peptide chains was studied in a cell-free system from rabbit reticulocytes, we obtained results which in several respects were opposite to those obtained with intact cells^{5,6,16}. Thus, reduced toxins, consisting of a mixture of A and B chains were much more potent than the intact toxins (Table 2). Experiments with the isolated chains showed that the inhibitory effect on protein synthesis is associated with the A chain of the toxins, whereas the isolated B chains were comparatively inactive. The results suggest that when the A chain is bound with a disulphide bridge to the B chain, it is prevented from inhibiting protein synthesis in a cell-free system. Possibly, the moderate inhibition observed when intact toxin is added (Table 2) may be accounted for by the fact that the cell-free protein

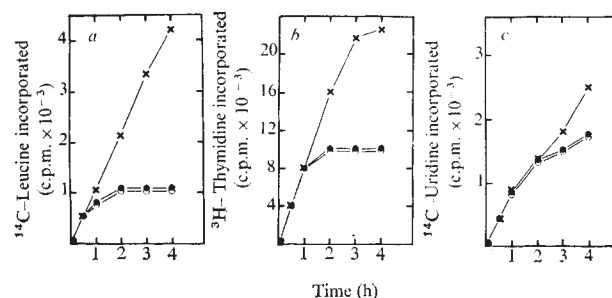


Fig. 2 Effect of abrin and ricin on the synthesis of protein (a), DNA (b), and RNA (c) in Ehrlich ascites cells *in vitro*. Abrin and ricin were extracted and purified to homogeneity as described elsewhere^{5,6}. To 1-ml samples of Ehrlich ascites cells, washed and suspended in incubation medium as described elsewhere¹⁹, were added 0.5 µCi ¹⁴C-leucine (specific activity 331 Ci mol⁻¹), 10 µCi methyl-³H-thymidine (specific activity 24.5 Ci mol⁻¹) or 0.5 µCi ¹⁴C-uridine (specific activity 505 Ci mol⁻¹) as indicated. The samples were incubated at 37° C, aliquots were removed after different times and the acid-precipitable radioactivity was measured as described elsewhere¹⁹ with the exception that in the case of (c), the aliquots were poured directly into cold 5% trichloroacetic acid rather than pretreated with 0.1 N NaOH, ×, Control; ○, 0.1 µg abrin; ●, 0.1 µg ricin.

synthesising system contains a low concentration of 2-mercaptoethanol capable of liberating small amounts of A chain.

Since a complete toxin consisting of both A and B chains is required for activity on whole cells, it is clear that the B chain must have a specific function. Our recent experiments indicate that the B chain is necessary for binding the toxins to the cell surface^{5,6}. In indirect haemagglutination experiments, where erythrocytes, pretreated with isolated B chain (or intact toxin), were exposed to an antiserum against the toxin (Table 2), the cells agglutinated, indicating that the antibody molecules form bridges between the cells with anchor points in the B chains or intact toxin molecules bound to the cell surface. Under similar conditions, pretreatment of the cells with A chain did not result in agglutination with antiserum, and we have been able to demonstrate this in a more direct way. Sepharose particles possessing covalently bound intact toxins or isolated B chains will bind erythrocytes, whereas particles with bound A chains will not (Fig. 3).

Binding to surface receptors

The B chains of the toxins probably bind to galactose-containing receptors on the cell surface. We found that the haemagglutination by antisera of erythrocytes pretreated with B chains could be inhibited in the presence of galactose or lactose⁷. Furthermore, in the presence of lactose no erythrocytes became bound to Sepharose particles containing covalently bound toxins (Fig. 3). Also, the ability of abrin and ricin to inhibit protein synthesis in Ehrlich ascites cells is strongly reduced

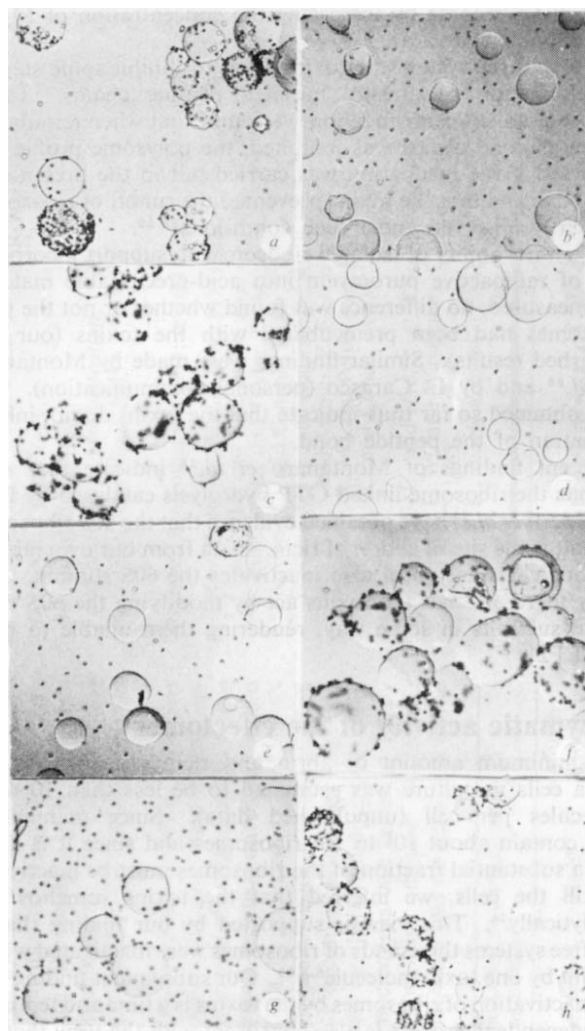


Fig. 3 Binding of erythrocytes to Sepharose particles with covalently bound toxins or B chains. Abrin and ricin or their constituent peptide chains were linked covalently to cyanogen bromide-activated Sepharose particles, (Pharmacia, Uppsala, Sweden) 10 mM lactose was present during the binding procedure to prevent the toxins from becoming bound by their specific binding sites to galactose residues of Sepharose. Sepharose was washed several times at high and low pH and with 0.1 M lactose to remove unbound material. It was then washed extensively with 0.1 M sodium phosphate (pH 7.1) containing 0.14 M NaCl and subsequently it was resuspended in the same buffer. One drop was mixed with about 10^7 human erythrocytes and mixed carefully by gentle shaking. Where indicated, 10 mM lactose was added. The mixture was examined under the microscope. *a*, Abrin; *b*, abrin (lactose); *c*, ricin; *d*, ricin (lactose); *e*, abrin A chain; *f*, abrin B chain; *g*, ricin A chain; *h*, ricin B chain.

when lactose is present in the culture medium (Fig. 4). In the presence of 10 mM lactose about twice as much abrin was necessary to reduce the protein synthesis by 50% as when lactose was omitted, and in the case of ricin about 30 times the concentration of toxin was required to obtain the same toxic effect as in the absence of lactose. Similar data have been obtained with ricin by other authors¹⁷. Evidence that only the B chain is involved in the binding of the toxins to galactose was obtained in experiments where we applied isolated chains of the toxins to a column of Sepharose 4B (which contains galactose residues). In this case the A chain passed through the column, whereas the B chain was retained and could be eluted with galactose⁶. The ability of the toxins to bind to Sepharose columns has been utilised in several laboratories for the purpose of purification of the toxins^{5,6,10,17}.

The toxins seem to have one binding site for galactose-containing carbohydrates, whereas the nontoxic agglutinins

present in the same seeds have two binding sites⁷. Thus we found in equilibrium dialysis experiments that each toxin molecule binds one molecule of lactose, whereas the agglutinins bind two lactose molecules per molecule. Abrin and ricin may therefore be considered as monovalent lectins. The binding constants were 8×10^3 l mol⁻¹ (abrin) and 1.5×10^4 l mol⁻¹ (ricin).

On the surface of different cells about 1×10^7 to 2×10^7 sites to which the toxins can bind seem to be present^{17,18}. The presence of a higher number of sites on the surface of malignant cells than on their normal counterparts may in part explain why abrin and ricin seem to be more toxic to malignant than to normal cells¹⁸.

Penetration of the toxins into cells

Since the B chains seem to bind the toxins to the cell surface we shall call this chain the 'haptomer' (Fig. 1). The A chain which carries the toxic activity, is denoted as the 'effectomer'. It is likely that after the toxins are bound to the cell surface by the haptomer, the A chain (or possibly the whole toxin) is transported into the cytoplasm to its site of action, the ribosomes (see below). It should be noted that whereas in a cell-free system protein synthesis is inhibited immediately after addition of the toxins⁵, we always found a lag of at least 30 min (at 37° C) when cells in culture were treated with the toxins, even

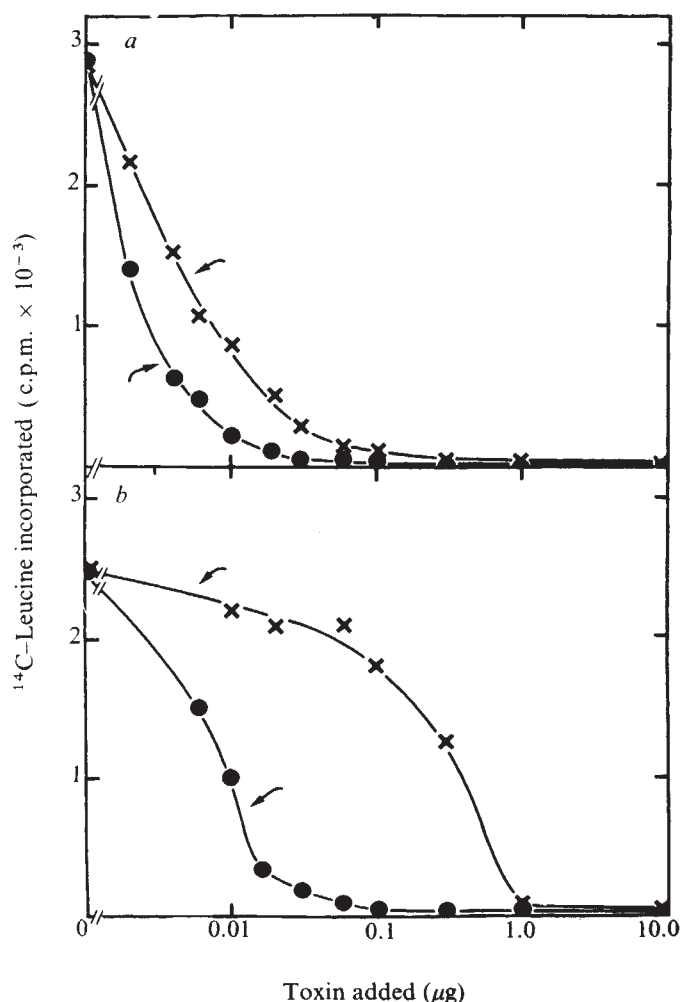


Fig. 4 Effect of lactose on the ability of abrin (*a*) and ricin (*b*) to inhibit protein synthesis in Ehrlich ascites cells. To 1-ml samples of a solution of Ehrlich ascites cells were added increasing amounts of toxin as indicated in the absence (●), and presence (×) of 10 mM lactose. The samples were incubated as in Fig. 2a and after 1 h, 0.5 μCi of ¹⁴C-leucine was added to each tube. The samples were further incubated for one more hour. Then aliquots were removed from each sample and the acid-precipitable radioactivity was measured.

Table 3 Protective effect of antitoxins added to Ehrlich ascites cells at different times after abrin and ricin

Time of toxin interaction with the cells before addition of antitoxin min	Inhibition of protein synthesis	
	Abrin %*	Ricin %*
0	0	0
5	33	11
10	67	44
30	92	78
60	100	100

To 1 ml samples of a suspension of Ehrlich ascites cells prepared as in Fig. 2a was added 1.0 µg of abrin and ricin. After incubation at 20° C for the indicated periods of time, a neutralising amount of antiabrin and antiricin was added. The samples were incubated at 20° C for a total of 60 min, then the incubation was continued at 37° C for 2 h to enable the effect of the toxins to develop (see Fig. 2). ¹⁴C-Leucine (0.5 µCi) was then added and the acid-precipitable radioactivity was measured as in Fig. 2a.

* Percentage reduction in radioactivity incorporated (control: 4,500 c.p.m.).

when the toxin concentration of the medium was as high as 100 µg ml⁻¹ (ref. 19). This lag is greater at lower temperatures (being 6 h at 20° C). Since the binding of the toxins to the cell surface seems to be rapid, it seems that the transportation of the toxin (or the free A chain) into the cytoplasm accounts for most of the lag. Our finding (Table 3) that the toxic effect can be counteracted by antitoxins only during the first 30 min of the 6 h lag at 20° C, indicates that during the remaining 5.5 h the toxins are not exposed on the outside of the cells.

They are possibly engulfed by endocytotic vesicles from which they are slowly liberated into the cytoplasm. It is generally believed that such vesicles merge with lysosomes. Our recent finding that the toxins are extremely resistant to proteolytic enzymes may be significant in this respect (S. O., K. R., and A. P., in preparation). The view that the cellular uptake of abrin and ricin involves endocytosis is supported by our finding that these toxins are unable to inhibit protein synthesis in reticulocytes which have little or no endocytotic activity¹⁹, even though the toxins bind readily to the surface of these cells. It is interesting to note that many viruses are taken up by a similar mechanism in that they bind to specific receptors on the surface of susceptible cells (viroplexis) and are then transported into the cytoplasm by endocytotic activity.

Mechanism of protein synthesis inhibition

The A chains of abrin and ricin have an immediate inhibitory effect on protein synthesis in a cell-free system from rabbit reticulocytes^{5,6}. To see whether abrin and ricin act on the ribosomes or on some soluble factors, we fractionated the lysate into ribosomes and a postribosomal supernatant, which were incubated separately with and without A chains from abrin and ricin²⁰. After incubation, the toxins were inactivated by addition of sufficient amounts of specific antitoxins. Subsequently, the ribosomes and the supernatants were recombined and a cell-free protein synthesising system was prepared. When the ribosomes had been pretreated with the toxins, virtually no incorporation of labelled amino acids into protein occurred, whereas pretreatment of the supernatant did not inhibit the amino acid incorporation. The fact that abrin and ricin inactivated isolated ribosomes indicate that a cofactor is not necessary. Our results indicate that only the eukaryotic type of ribosome is susceptible to the toxins, as protein synthesis in a cell-free system from *Escherichia coli*²⁰ was not reduced, whereas the incorporation of labelled amino acids into proteins in a cell-free system from rat liver²⁰ and wheat germ (our unpublished work) was strongly inhibited.

Not only protein synthesis directed by natural messengers, but also poly(U)-directed polymerisation of labelled phenylalanine, is inhibited²¹. The inhibition was strongest in the presence of a low concentration of Mg²⁺ ions (4 mM) and could

be partly overcome by increasing the concentration of MgCl₂ (our unpublished data).

In a cell-free system the toxins seem to inhibit some step in the elongation of already initiated peptide chains. Using sucrose gradient centrifugation we found that when reinitiation of new peptide chains was inhibited, the polysome profile was preserved if the incubation was carried out in the presence of the toxins and that the toxins prevented the runoff of ribosomes ordinarily observed under such conditions^{21,22}.

When the ability of isolated ribosomes to support incorporation of radioactive puromycin into acid-precipitable material was measured, no difference was found whether or not the pure ribosomes had been preincubated with the toxins (our unpublished results). Similar findings were made by Montanaro *et al.*²³ and by L. Carasco (personal communication). The data obtained so far thus indicate that the toxins do not inhibit formation of the peptide bond.

Recent findings of Montanaro *et al.*²³ indicate that ricin inhibits the ribosome-linked GTP hydrolysis catalysed by EF2, and Sperti *et al.*²⁴ have obtained evidence that the 60S ribosomal subunit is the site of action of ricin. Data from our own laboratory indicate that abrin also inactivates the 60S subunit. It is likely therefore that the toxins act by modifying the 60S ribosomal subunits in some way, rendering them unable to react with EF2.

Enzymatic activity of the effectomer

The minimum amount of abrin and ricin necessary to kill HeLa cells in culture was estimated to be less than 10 toxin molecules per cell (unpublished data). Since mammalian cells contain about 10⁵ to 10⁶ ribosomes and since it is likely that a substantial fraction of the ribosomes must be inactivated to kill the cells, we inferred that the toxins somehow act catalytically¹⁶. This view is supported by our finding that in cell-free systems thousands of ribosomes were inactivated within 30 min by one toxin molecule^{5,6,16}. Our subsequent finding that the inactivation of ribosomes by the toxins is a time and temperature-dependent process is also consistent with the view that the toxins possess enzymatic activity²⁰. The possibility that the A chains of the toxins activate an enzyme which then inactivates the ribosomes, is unlikely since the A chains inactivate isolated ribosomes in simple buffer solutions²⁰. It is not known whether the toxins are specific proteases or specific RNases inactivating eukaryotic ribosomes in a similar way as colicin E3 inactivates bacterial ribosomes (for review see ref. 1).

Implications

The finding that the toxic plant proteins abrin and ricin consist of a haptomer moiety responsible for the binding of the toxin to surface receptors of susceptible cells and an effector moiety responsible for the specific toxic action, seem to be of considerable interest. Thus, it has previously been shown that diphtheria toxin consists of an enzymatically active A chain which inhibits protein synthesis by inactivating the elongation factor EF2 and a B chain which binds the toxin to specific sites on the surface of susceptible cells (for review see ref. 25). Evidence is now accumulating that cholera toxin²⁶, botulinus toxin²⁷ and tetanus toxin²⁸ may similarly consist of a haptomer and an effectomer moiety. Also it seems possible that uptake by endocytosis after specific binding to the cell surface may be widespread in nature. It is an interesting possibility that abrin and ricin pass the cell membrane by routes originally developed for the uptake of physiological proteins, like certain hormones and growth factors.

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Role of myosin light chains in calcium regulation

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The evidence that rabbit DTNB light chains bind to 'desensitised' scallop myosin and restore calcium sensitivity, that is, they functionally replace the scallop EDTA light chain, may suggest that these light chains are involved in some type of calcium regulatory mechanism in rabbit myosin in vivo.

In skeletal muscle, contraction is regulated by the interaction of calcium ions with the specific protein troponin, which is attached to the actin filaments¹. In molluscan muscles² and in some primitive invertebrates³, on the other hand, contraction is regulated by the interaction of calcium with myosin. Molluscan myosin preparations bind calcium and their ATPase activity in the presence of pure actin is calcium dependent². The calcium dependence of the actin-activated ATPase activity of scallop myosin requires the presence of a specific light chain, called the EDTA light chain because it is released from myosin if the divalent cation concentration of the medium is lowered by the addition of EDTA⁴. The release of the light chain results in a loss of calcium sensitivity, that is, the myosin is 'desensitised' and the calcium requirement for the actin activated ATPase activity is abolished. Recombination of the EDTA light chain with the desensitised myosin in the presence of divalent cations restores calcium sensitivity, showing that the light chain functions as a regulatory subunit.

Comparative studies on the calcium regulatory systems of a wide variety of species³ have revealed that the muscles of some invertebrate species contain both myosin-linked and actin-linked calcium regulation. These results prompted an investigation of a possible role in calcium regulation of the myosin light chains in other species, such as vertebrates, whose muscles seem only to show actin-linked calcium regulation.

The number, size and chemical characteristics of myosin light chains from a variety of vertebrate species and different muscle types are rather diverse⁵⁻¹³ and, as yet, there is no unambiguous demonstration of the role of the subunits in the functions of the myosin molecule. Rabbit skeletal

myosin, the most extensively studied, contains three light chain components and these may be divided, on the basis of chemical studies¹⁴ into two distinct classes—(1) the so-called 'alkali' light chains¹⁵ with molecular weights of 21,000 and 17,000 which have extensive sequence homology¹⁶ and have been implicated in regulation of the enzymatic activity of the myosin^{17,18} and (2) the DTNB light chain with a molecular weight of about 19,000, which is selectively released from myosin by treatment with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB)^{7,15}. In view of the evidence¹⁴ that this DTNB light chain is not required for the hydrolytic activity of the myosin, since its selective removal by treatment with DTNB does not significantly alter the ATPase activity of the residual myosin, a possible role for this light chain in calcium regulation was investigated.

DTNB light chain and rabbit myosin

Treatment of rabbit skeletal myosin with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB)¹⁵, which blocks about 80% of the myosin thiol groups, leads to the selective release of 50–60%, (about 1 mol) of the DTNB light chains. Treatment with EDTA alone⁴ does not remove the DTNB light chain. The loss of 1 mol of DTNB light chain does not significantly alter the properties of the residual myosin; it has little effect on the ATPase activity or on the ability of the myosin to interact with actin, and a calcium-sensitive complex is formed when this myosin is mixed with actin containing the calcium regulatory proteins troponin and tropomyosin. Similarly, if subfragment 1 is prepared from DTNB-treated myosin by papain cleavage¹⁹, no intact DTNB light chains are detectable by polyacrylamide gel electrophoresis in the presence of SDS (Fig. 1). Double reciprocal plots of the actin-activated ATPase of this subfragment²⁰ yields a value for $V_{\max}$ ($12 \mu\text{mol ATP min}^{-1} \text{mg}^{-1}$) similar to that obtained with subfragment 1 prepared from untreated myosin. This subfragment also interacts with actin containing tropomyosin and troponin to form a calcium-sensitive complex. The functional significance of the DTNB light chain is therefore unclear and its presence is apparently not required for the normal biochemical functions of the

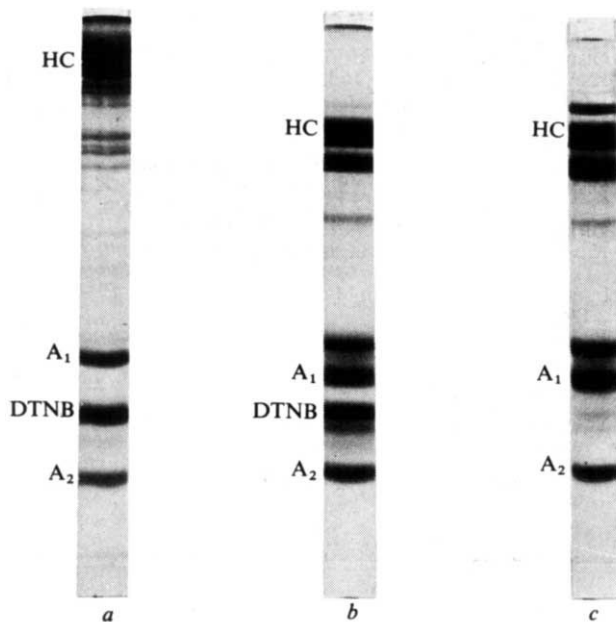


Fig. 1 10% SDS polyacrylamide gel electrophoresis²⁹ of: *a*, rabbit myosin; *b*, subfragment 1 prepared from untreated rabbit myosin; *c*, subfragment 1 prepared from DTNB-treated rabbit myosin. Buffer system used was 100 mM Tris bicine (pH 8.3), 0.1% SDS and the gels were stained with 0.5% Coomassie brilliant blue. HC, heavy chains of myosin and subfragment 1; A₁, 'alkali' 1 light chain; DTNB, DTNB light chain; A₂, 'alkali' 2 light chain.

myosin. It is difficult to accept that this light chain is a tightly bound contaminant of the myosin, especially in view of the stoichiometric amounts found in myosins from several species¹⁰. An alternative explanation is that the DTNB light chains in the intact muscle may have a function that has been lost during extraction and purification of the myosin; for example, it may, like the EDTA light chain in molluscan myosin, be involved in some type of calcium regulation. To explore this possibility, the interaction of the DTNB light chain with 'desensitised' molluscan myosin preparations was studied.

DTNB light chain and 'desensitised' molluscan myosin

Treatment of scallop myofibrils, actomyosin or myosin with 10 mM EDTA (ref. 4) leads to the release of 1 mol of the EDTA light chain and to a loss of calcium sensitivity, that is, the preparations are 'desensitised' and the calcium requirement for the actin-activated ATPase activity of the myosin is abolished. Rabbit DTNB light chains were added to preparations of desensitised scallop myofibrils, actomyosin and myosin and the mixtures were washed extensively to remove nonspecifically bound light chains. Polyacrylamide gel electrophoresis in the presence of SDS of these preparations (Fig. 2) shows that the DTNB light chain combined readily with these desensitised preparations. In the case of the desensitised myofibril and actomyosin preparations, the binding of the DTNB light chain always leads to a restoration of calcium sensitivity (Table 1) that is, the DTNB light chain can replace the scallop EDTA light chain. In the case of purified myosin preparations, however, the results are somewhat more complicated. Although the DTNB light chain always binds to the desensitised myosin preparations (Fig. 2), its ability to restore calcium sensitivity to these preparations is rather variable. The results in Table 2 illustrate that the DTNB light chain can in some cases, fully restore calcium sensitivity to the desensitised myosin. In certain other preparations, however, even though the DTNB

light chain is bound, the myosin remains completely insensitive to calcium. These desensitised myosin preparations, however, become calcium sensitive when combined with the scallop EDTA light chain. This lack of calcium sensitivity when the DTNB light chain binds to certain desensitised myosin preparations must be due to rather subtle alterations in the structure of the myosin, and indicates that the DTNB light chain requires a rather more 'specific myosin configuration' than the EDTA light chain does, in order to bind in the correct position and restore calcium sensitivity. The reasons for the variability in resensitising myosin with the DTNB light chain are at present unclear. Since the desensitised myofibril and actomyosin preparations can always be resensitised by the addition of the DTNB light chain, the presence of actin in these preparations probably stabilises the structure of the desensitised myosin. We have no evidence to suggest that the DTNB light chain interacts with either the actin or tropomyosin in these preparations.

To measure directly the amount of DTNB light chain bound to the myosin in these desensitised preparations, the light chains were radioactively labelled by alkylating their thiol groups with ¹⁴C-labelled iodoacetic acid. This treatment has no effect on the ability of the light chain to bind and to restore calcium sensitivity. Using these labelled DTNB light chains, it was found that calcium sensitivity was completely restored when about 1 mol of light chain is bound per myosin molecule, and the extent of restoration of calcium sensitivity correlates approximately with the amount of light chain bound (Tables 1 and 2). Only 1 mol of DTNB light chains is bound, even when a large excess of light chain is added. These values are consistent with our previous findings²¹, that 1 mol of EDTA light chain is released when the myosin is desensitised by treatment with EDTA. These results and the fact that neither intact myofibrils nor myosin bind the DTNB light chain unless the EDTA light chain has been removed, further suggest that the DTNB light chain must bind at approximately the same position on the myosin heavy chains as the EDTA light chain.

Release of the EDTA light chain from scallop myofibril and myosin preparations decreases the amount of calcium bound by the myosin in these preparations by about 40%

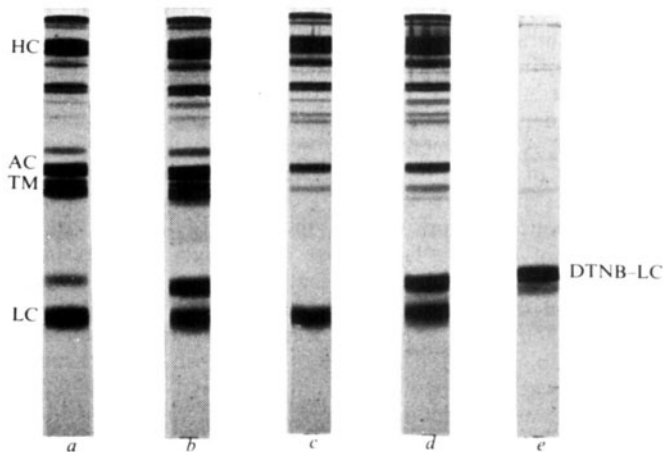


Fig. 2 10% SDS polyacrylamide gel electrophoresis demonstrating the binding of rabbit DTNB light chains to desensitised scallop myofibril and myosin preparations. *a*, Desensitised scallop myofibrils; *b*, desensitised myofibrils plus DTNB light chain; *c*, desensitised scallop myosin; *d*, desensitised scallop myosin plus DTNB light chain; *e*, DTNB light chain. The isolated rabbit DTNB light chain was added in large excess to the desensitised scallop preparations in 40 mM NaCl, 1 mM MgCl₂, 5 mM phosphate buffer (pH 7.0) and these preparations were washed extensively to remove the unbound light chains. HC, myosin heavy chains; AC, actin; TM, tropomyosin; LC, scallop light chains; DTNB-LC, rabbit DTNB light chain.

Table 1 Effect of the DTNB light chain on the ATPase activity of desensitised scallop myofibrils

¹⁴ C-DTNB light chain added (mol per mol myosin*)	¹⁴ C-DTNB light chain bound (mol per mol myosin*)	Actin-activated ATPase ($\mu\text{mol ATP mg}^{-1} \text{ min}^{-1}$ at 25° C)		Ratio EGTA/Ca ²⁺
		0.1 mM EGTA	0.1 mM Ca ²⁺	
Control	—	0.186	0.194	0.96
0.22	0.16	0.170	0.203	0.84
0.44	0.36	0.147	0.240	0.61
0.70	0.60	0.070	0.232	0.34
1.10	0.90	0.031	0.238	0.13
1.50	1.18	0.022	0.246	0.09
Native myofibrils	—	0.018	0.208	0.08

*Assuming that the myosin content of desensitised myofibrils is 65%, the molecular weight of the myosin is 425,000 and that of the DTNB light chain is 19,000.

The desensitised myofibrils were incubated for 10 min with increasing amounts of the ¹⁴C-labelled DTNB light chain in 40 mM NaCl, 1 mM MgCl₂, 5 mM phosphate (pH 7.0) and washed four times with 10 volumes of the same solution. Aliquots were taken for ATPase measurements⁴ and for liquid scintillation counting to determine the amount of light chain bound.

(ref. 4). Full calcium binding is regained when the EDTA light chain and the desensitised myosin are recombined (Table 3). When the DTNB light chain is combined with desensitised myofibril and myosin preparations, however, the amount of calcium bound is increased by only 10–20%. This rather lower figure raises the question, especially in view of the results in Table 1 and 2 which indicate that the DTNB light chain can fully restore calcium sensitivity to these preparations, whether there is a correlation between the restoration of calcium sensitivity and the amount of calcium bound. To answer this question, the ATPase activities and the amount of light chain bound by desensitised myofibrils were assayed after the calcium binding measurements. The results in Table 3 indicate that during our calcium binding procedure⁴ there was a slight decrease in the amount of DTNB light chain bound (0.89 mol light chain bound per mol myosin) and a decrease in calcium sensitivity (compare Table 1). Therefore, when the DTNB light chain combined with desensitised myofibrils, the restoration of calcium sensitivity seems to parallel the increase in the amount of calcium bound by these preparations.

The DTNB light chain is tightly associated with scallop myosin, since it is not released by repeated reprecipitation or under mild dissociation conditions. The binding of light chain to myosin heavy chain also seems to be specific for the DTNB light chain, since the other types of light chains, that is, the rabbit 'alkali' light chains isolated from DTNB-treated myosin by treatment with guanidine hydrochloride⁹ and purified by chromatography on DEAE-cellulose¹⁴ do not bind and do not confer calcium sensitivity to these desensitised preparations. To confirm this observation, rabbit myosin was treated with 5 M guanidine hydrochloride²² which dissociates the 'alkali' and DTNB light chains, and both

types of light chains were added to a desensitised scallop myofibril preparation, which selectively binds solely the DTNB light chain and restores calcium sensitivity. Preliminary evidence indicates that this type of 'regulatory' light chain may be a common feature of all myosins, for example, cardiac, gizzard smooth, lobster and frog striated muscle myosins all contain a light chain component that behaves, with respect to desensitised scallop muscle preparations, like the rabbit DTNB light chain. (J. K.-J., E. M. Szentkiralyi and A. G. Szent-Györgyi, manuscript in preparation).

Scallop EDTA light chain and rabbit myosin

Since the rabbit DTNB light chain combined with desensitised scallop myosin, it was natural to enquire whether the scallop EDTA light chain crossreacts with DTNB-treated rabbit myosin. To answer this question, EDTA light chains were radioactively labelled by iodinating their tyrosine residues with ¹²⁵I (ref. 24) under very mild oxidising conditions. These ¹²⁵I-labelled light chains function normally with desensitised scallop myosin and bind to DTNB-treated rabbit myosin (about 0.6 mol light chain bound per mol of myosin) but they have no effect on the actin-activated ATPase activity of rabbit myosin when mixed with actin in the presence and absence of calcium, that is, the myosin remains calcium insensitive. Native rabbit myosin binds less than 0.1 mol labelled EDTA light chain per mol myosin, which suggests that the DTNB light chain and EDTA light chain binding sites on this myosin are probably the same or in the same region of the myosin heavy chains. Since the EDTA and DTNB light chains are interchangeable in their respective myosins one might have supposed that these light

Table 2 Desensitised scallop myosin and rabbit DTNB light chain

¹⁴ C-DTNB light chain added (mol per mol myosin*)	¹⁴ C-DTNB light chain bound (mol per mol myosin*)	Actin-activated ATPase ($\mu\text{mol ATP mg}^{-1} \text{ min}^{-1}$)		Ratio EGTA/Ca ²⁺
		0.1 mM EGTA	0.1 mM Ca ²⁺	
Control	—	0.201	0.215	0.93
0.30	0.24	0.130	0.220	0.59
0.60	0.52	0.076	0.216	0.35
0.82	0.74	0.052	0.238	0.22
1.52	0.94	0.057	0.234	0.24
2.98	1.08	0.048	0.186	0.26
Native myosin	—	0.030	0.242	0.13

*Assuming that the molecular weight of scallop myosin is 425,000 and the DTNB light chain is 19,000.

Desensitised myosin was mixed with varying amounts of the light chain in 0.6 M NaCl, 1 mM MgCl₂, 10 mM phosphate (pH 7.0) and dialysed against 40 mM NaCl, 1 mM MgCl₂, 5 mM phosphate (pH 7.0). The precipitated myosin was washed extensively and then dissolved in 0.6 M NaCl (pH 7.0). For the ATPase measurements, the myosin was mixed with rabbit actin (2:1 w/w).

Table 3 Correlation between the restoration of calcium sensitivity and the calcium binding of scallop myofibrils

	Actin-activated ATPase ($\mu\text{mol ATP mg}^{-1} \text{ min}^{-1}$ at 25°C)		Ratio EGTA/ Ca^{2+}	Calcium binding $\mu\text{mol Ca per g muscle}$ at $10^{-6} \text{ M Ca}^{2+}$
	0.1 mM EGTA	0.1 mM Ca^{2+}		
Native myofibrils	0.020	0.281	0.07	2.05
Desensitised myofibrils	0.232	0.251	0.93	1.34
Desensitised myofibrils + scallop EDTA light chain	0.032	0.278	0.12	1.94
Desensitised myofibrils + DTNB light chain	0.093	0.262	0.35	1.62

Excess EDTA light chains and DTNB light chains were mixed with desensitised myofibrils as described in Table 1. The myofibrillar preparations were subjected to our calcium binding procedure⁴, then assayed for ATPase activity⁴ and the amount of bound DTNB light chain measured.

chains may have considerable chemical similarity. However, a comparison of their chemical structures as revealed by amino acid analyses and tryptic peptide maps^{4,14} indicates no obvious sequence homology. The question of whether these different light chains have highly conserved regions, which may be the heavy chain binding sites, will therefore require more extensive knowledge of their amino acid sequences, which is now under investigation.

Implications

Our knowledge about the role of the DTNB light chain in rabbit myosin is extremely limited. The evidence that rabbit DTNB light chains bind to desensitised scallop myosin and restore calcium sensitivity, that is, they functionally replace the scallop EDTA light chain, is the first indication of a possible functional role of these light chains in rabbit myosin. It may be that these light chains are involved in calcium regulation in rabbit muscle or they may be remnants of a myosin-linked regulatory system that has lost its ability to respond to calcium, due to alterations in the heavy chains. To decide definitely whether vertebrate muscles have a myosin-linked calcium regulatory system similar to that found in molluscan muscles, one must establish directly that these myosins are able to respond to calcium. The available biochemical evidence, however, indicates that purified vertebrate myosins bind rather small amounts of calcium (at 10^{-6} M free calcium) in the presence of physiological levels of free magnesium, and show a calcium insensitive ATPase activity when mixed with pure actin^{1,24}. This does not however, exclude the possibility that the myosin binds calcium *in vivo*, since it can be argued that during extraction and purification, this ability has been irreversibly lost. Some support for such a speculation may be provided by recent X-ray diffraction studies^{25,26} on stretched frog muscles, which indicate but do not prove, that the myosin cross bridges may be able to respond to changes in the level of free calcium in the muscle. The evidence for and against some type of calcium control by the myosin in vertebrate muscles has been extensively discussed in a recent review of Weber and Murray²⁷, who conclude that the question of whether there is some type of control mechanism on the myosin in these muscles remains unresolved.

The evidence presented here indicates that the DTNB light chain and EDTA light chain seem to be interchangeable. Even if their overall functions are similar, that is, switching the myosin 'on' in response to the release of calcium during activation, we cannot exclude the possibility that the exact mechanisms involved maybe rather different. For example, in molluscan muscles, calcium released upon activation acts directly on the myosin², whereas in rabbit muscle, calcium may act indirectly on the myosin by activating a specific calcium requiring protein kinase²⁸, known

to be able to phosphorylate selectively the DTNB light chain, and thus under *in vivo* conditions this light chain may be phosphorylated which may result in the myosin being switched 'on'²⁸.

To answer the questions of whether vertebrate myosins contain a regulatory control mechanism and what is the nature of such a mechanism, and to establish the role played by the DTNB light chains, therefore, requires further work specifically with the intact muscle.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Structure of the visible Jovian clouds

DURING the 1972 apparition of Jupiter we studied hydrogen quadrupole line intensities at the centre of the planetary disk, by observing the S(1) lines of the H_2 (3-0) and (4-0) quadrupole bands which are respectively centred at 12268.8 and 15704.01 cm^{-1} . These observations show that the abundance of hydrogen detected at these frequencies is extremely variable because of rapid changes in the structure of the visible clouds, which are confirmed by photographs of the planet.

We observed with the Coude spectrograph of the 61-cm (24-inch) telescope at the Table Mountain Observatory. Spectra were obtained at 1.6 $\text{\AA mm}^{-1}$ reciprocal dispersion in the (4-0) band and 2 $\text{\AA mm}^{-1}$ in the (3-0) band, and were recorded on photographic plates using a single stage S-25 image intensifier tube. The plates were traced with a digital microphotometer and the digital data were reduced to intensity tracings by a computer routine using conventional techniques of photometry. The photometric calibration was checked by measuring, on each tracing, the equivalent widths of several Fraunhofer lines selected to match approximately the intensities and frequencies of the Jovian H_2 lines. The measured intensities of the Fraunhofer lines were systematically low on the tracings and linear least-squares fits were used to correct the measured H_2 lines. The error estimates of one standard deviation (σ) computed from these correction processes are given with the individual measurements.

In Figs 1 and 2 we show the measured equivalent widths of the (3-0)S(1) and (4-0)S(1) hydrogen quadrupole lines as a function of the SYSTEM I longitude. The magnitude of the (4-0)S(1) equivalent widths (EW) varies from 4.2 ± 0.7 to 14.9 ± 2.4 m $\text{\AA}$. This is in contrast to the previously published observations^{1,2} which had all centred at about 10 m $\text{\AA}$ for the

EW and seemed to be independent of both the observer and the terrestrial conditions. The (3-0)S(1) equivalent widths show a similar variation at 34.5 ± 11.3 to 56.9 ± 6 m $\text{\AA}$.

The measurements have been interpreted by constructing a realistic model of the Jovian atmosphere in which all the processes of radiative transfer have been accurately considered¹. Hydrogen quadrupole lines exhibit the phenomenon of collision narrowing before the more usual pressure broadening occurs, and so it is important that the correct line shape is used in an analysis³. We have used the Galatry profile which accurately represents the quadrupole line shape. The nominal model of the Jovian atmosphere proposed by Divine⁴, and an H/H_e ratio of 6:1 (ref. 5), which are both consistent with present knowledge, are used as a basis for this analysis.

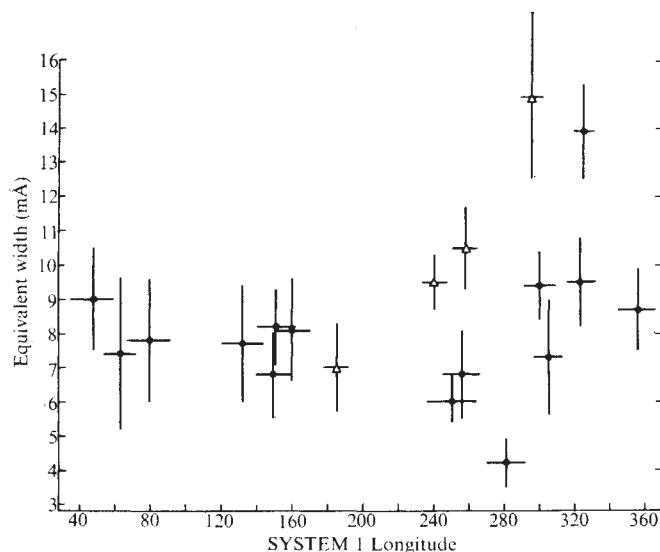


Fig. 1 The variation of the equivalent width of the (4-0)S(1) quadrupole line in the Jupiter spectrum with the SYSTEM I longitude. Solid circles, observations made June 14-30, 1972; triangles, August 17-19, 1972.

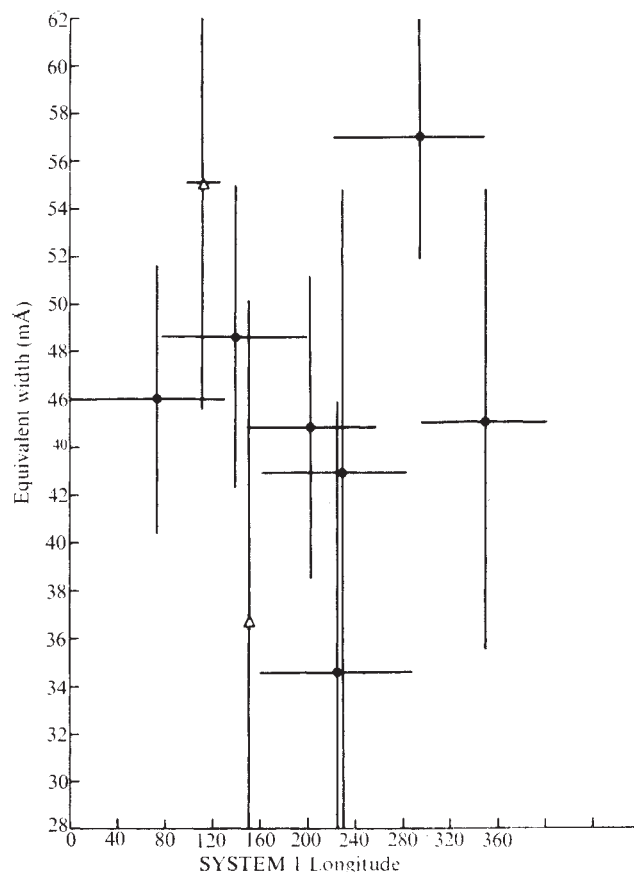


Fig. 2 As Fig. 1 but for the (3-0)S(1) line. Solid circles, June 6-28, 1972; triangles, August 16-18, 1972.

In the equatorial region of Jupiter a structure of two distinct cloud layers is consistent with all observations⁵. The upper cloud may be composed of ammonia crystals, but the composition of the lower layers is unknown¹. The upper cloud layer is optically thin¹ and we found that it has a negligible effect on the equivalent widths which we observed at the centre of the disk. The lower cloud is optically thick and behaves like a reflecting surface. Consequently, the changes in the derived effective pressure at the level of formation of weak lines, such as the (4-0)S(1) line, will indicate the variability of the top of this dense, lower cloud.

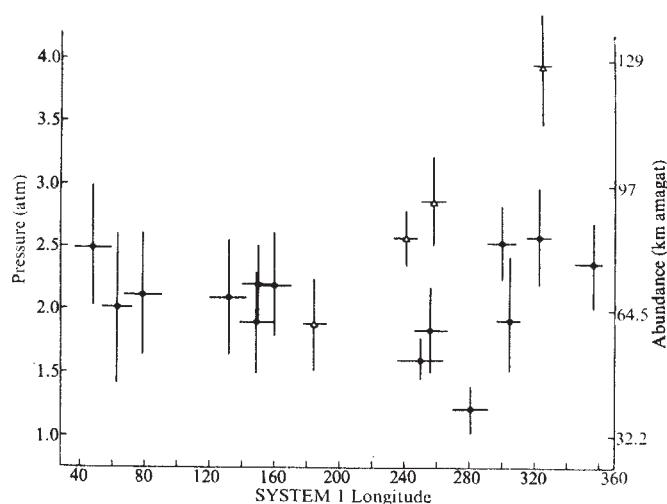


Fig. 3 The variation of the effective pressure at the level of the formation, and the relative abundance of hydrogen, as a function of the SYSTEM I longitude derived from observations of the (4-0)S(1) quadrupole line (Fig. 1).

From the (4-0)S(1) observations we have derived a possible variation of 1.22 ± 0.18 to 4.03 ± 0.93 atm in the effective pressure with a corresponding relative abundance of 39.4 ± 5.9 to 145 ± 30 km amagat (Fig. 3). (1 amagat is the unit of density or volume referring to a gas at 0°C and 1 atm pressure). The analysis of the (3-0)S(1) indicated similar variations of 1.045 ± 0.41 to 2.29 ± 0.51 atmospheres for the effective pressure with the relative abundance varying between 33.85 ± 3.15 and 74.5 ± 16.5 km amagat (Fig. 4). This is a stronger, saturated line which is less sensitive to changes of cloud structure, than the weak (4-0)S(1). These results indicate the futility of quoting a single value for either the effective pressure or the relative abundance derived from spectroscopic observations of a dynamically active planet such as Jupiter.

There are two significant features in the observations illustrated in Figs 1 and 2. First, for the SYSTEM I longitude in the range $160^\circ \leq \lambda \leq 280^\circ$ the observations carried out during June 1972 show a significant decrease in all of the measured line strengths. For the (4-0)S(1) line this requires the effective pressure at the level of line formation (and therefore at the position of the cloud top) to decrease from $\sim 2 \pm 0.5$ to $\sim 1.25 \pm 0.2$ atm and the hydrogen abundance varies between 68 ± 15 – 39 ± 13 km amagat. For the (3-0)S(1) line the pressure varies between $\sim 1.6 \pm 0.5$ – $\sim 1 \pm 0.4$ atm and the abundance

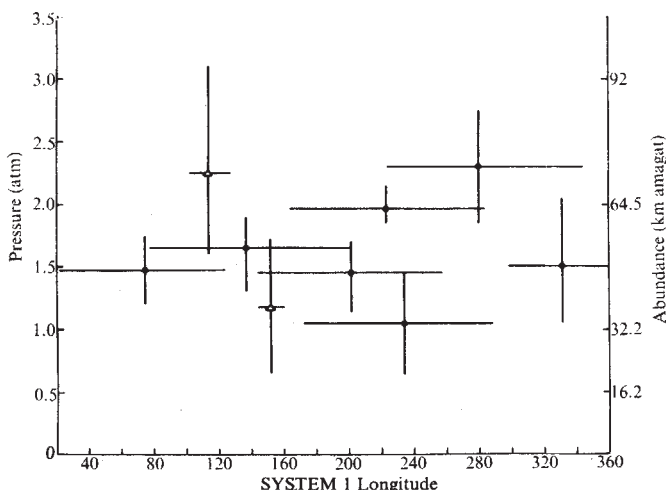


Fig. 4 As Fig. 3 but for the observations of the (3-0)S(1) quadrupole lines (Fig. 2).

varies between $\sim 54 \pm 16$ – $\sim 33 \pm 13$ km amagat. If these variations are interpreted in terms of changes in the position of the top of the dense, lower cloud, the effective pressure derived from the (4-0)S(1) observations requires this cloud to rise by ~ 25 km from the 2 atm level, during the observational period.

Photographs from the Lowell Observatory International Planetary Patrol, taken at times corresponding to our spectroscopic observations, show significant changes with SYSTEM I longitude of the detailed appearance of the broad equatorial zone. Keay *et al.*⁷ have detected temperature variations greater than 40 K in the equatorial zone and have found a strong correlation between temperature and the colours of cloud features in the zones. This increases our confidence in both the detection of fluctuations in the apparent molecular hydrogen abundance and in the interpretations of variations, through a large range of temperature and pressure, in the position of the top of the lower cloud.

With this information we can estimate an upper limit to the vertical velocity of the atmosphere in the neighbourhood of the cloud tops during the observation period. We are assuming that our observations refer to some portion of the planet as it rotates. Hide⁶ suggests an upper limit on the vertical velocity W (m s^{-1}) of a fluid element may be derived from the equation

$$W \leq U^2 d / f L^2 \quad (1)$$

where f is the vertical component of rotation ($W \cdot 10^{-4} \text{ s}^{-1}$). As our observations refer to the equatorial zone we used values of $L \sim 6 \times 10^6$ m for its width and $U \sim 100 \text{ m s}^{-1}$ for a typical horizontal velocity⁵. Inserting a value of $d \sim 25$ km for the height through which the cloud top has moved, equation (1) gives a reasonable estimate of the upward velocity necessary to account for this change in cloud structure:

$$\sim \leq 7.5 \text{ cm s}^{-1} \quad (2)$$

Hide⁶ has suggested a value of $\sim \leq 10 \text{ cm s}^{-1}$ by observing the changes in colour brought about by the supposed vertical mixing in the atmosphere.

The second significant feature of the observations occurred during August 1972 when an increase in line strength was measured for all lines over the SYSTEM I longitude range of 180 – 320° . For the (4-0)S(1) line this means that the effective pressure must increase (the level of the lower cloud must decrease) from 1.38 ± 0.77 to 4.43 ± 0.93 atm and the abundance must increase from 61.25 ± 11.75 to 145 ± 30 km amagat. The corresponding changes for the less sensitive (3-0)S(1) line are from approximately 1.18 ± 0.52 to 2.26 ± 0.89 atm and 38.25 ± 16.75 to 73.75 ± 29.25 km amagat. Assuming that we are referring to a single portion of the disk this variation requires the lower cloud to decrease in height by 25 km from ~ 1.38 – 4.43 atm level during the observational period of two Earth days. From equation (1), the downward velocity, W , will be $\leq 7.5 \text{ cm s}^{-1}$.

This patrol of H_2 quadrupole lines has shown that the abundance of hydrogen and the level of formation of these quadrupole lines is extremely variable. The observations of Keay *et al.*⁷ support our conclusions.

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Model for the binary X-ray source 2U1700-37

Six X-ray sources have been found which show a periodic variability in their X-ray flux which may indicate orbital motion of a binary system¹.

Two of these sources, Her X-1 and Cen X-3, have pulsed emission with periods respectively of 1.24 s and 4.8 s. From the variation of the pulsed emission period due to the Doppler effect during the orbital motion it is possible to deduce some parameters of the orbit and some physical properties of the system.

A possible model for these X-ray sources is the following: a binary system is made up of a neutron star (perhaps a black hole in the case of Cyg X-1), which is the X-ray source and a star which fills its Roche lobe. The X-ray luminosity is provided by the mass transfer from the star through the internal Lagrangian point and that is accreted by the neutron star.

In the case of Her X-1 and Cen X-3, if the neutron star is rotating and has a strong magnetic field, then the matter is accreted chiefly by the magnetic poles producing hot spots that emit X rays and consequently a pulsed emission. This has been studied in some detail by Davidson and Ostriker² and Gnedin and Sunyaev³.

The evolutionary picture of such a system, with special emphasis on Cen X-3, has been given by Van den Heuvel⁴, who has studied the evolution of a close binary system in which a large mass transfer occurs.

The matter transferred from the primary star to the neutron star (X-ray source) is likely to have angular momentum. Therefore a rotating disk will be formed, which gradually spirals inwards as turbulent viscosity transports its angular momentum outward. The spectrum of the radiation emitted by the disk was calculated by Pringle and Rees⁵ and Shakura⁶.

The model explains reasonably well the properties of Her X-1, Cen X-3, Cyg X-1 and 2U0900–40 (ref. 7) but it fails in the case of 2U1700–37 (see ref. 1).

Here I suggest that in the case of the source 2U1700–37, the primary star, an 07f type star (HD153919, refs 1, 7 and 8) is not filling its Roche lobe and that the matter which is accreted by the neutron star is due to a strong stellar wind from the primary star.

If the primary star fills the Roche lobe, we have a relation between ψ , which is the half angle in the orbital plane subtended by the critical lobe as seen from the X-ray source and the ratio M_s/M_x , where M_s is the mass of the primary star and M_x that of the X-ray source.

I first assume that the orbital inclination angle i is 90° . Then, $\psi/180^\circ$ is the fraction of the period during which the X-ray source is eclipsed. From the data given by Jones *et al.*¹, $\psi \sim 58^\circ$. I note that 2U1700–37 is the source which shows the greatest occultation time, almost one-third of the period.

If $\psi \sim 58^\circ$ the mass ratio is $M_s/M_x \sim 240$.

On the other hand, recent measurements of radial velocity of HD153919 by Van den Heuvel⁹ show a nearly sinusoidal variation with an amplitude of 23 km s⁻¹. Since the period is 3.41 d (ref. 1), the mass function is:

$$\frac{M_x \sin^3 i}{(1 + M_s/M_x)^2} = 4.3 \times 10^{-3} M_\odot \quad (1)$$

With the mass ratio calculated above and $i = 90^\circ$, and using equation (1): $M_x \sim 250 M_\odot$ and $M_s \sim 6 \times 10^4 M_\odot$, which are very implausible values. The results are even worse when $i < 90^\circ$. I conclude that the primary star does not fill the Roche lobe.

I now assume that the primary star has a strong stellar wind. The primary star is an 07f type and thus has enough ultraviolet

radiation to ionise the wind well beyond the neutron star orbit and therefore the accreted material consists mainly of protons and electrons.

I suggest that the occultation of the X-ray source is not only due to the eclipse but also due to the Thompson scattering of the X rays in the extended envelope of the primary star. This seems to be supported by the gradual transition from the visibility to the occulted phase and by the variability on time scales of minutes¹ which can reflect irregularities in the wind, that is partially driven by the X-ray source (J. Aarons, unpublished).

A similar analysis was made by Pringle¹⁰ in the case of Her X-1. In this case, since the primary star is likely to be a FO type, ionisation of the envelope is negligible. There is only a kind of 'Stromgren surface' produced by the soft X rays emitted by the neutron star.

To make some crude calculations of the stellar wind parameters, I assume a constant wind velocity V . In this case, matter conservation requires a wind density $n(r)$, which falls as the inverse square of the distance r from the star, namely,

$$n(r) = \frac{K}{r^2} \quad (2)$$

where K is a constant to be calculated. I further assume that $i = 90^\circ$.

Then, the optical depth in a given direction θ is

$$\tau(\theta) = \int_0^\infty n(x, \theta) \sigma_T dx \quad (3)$$

where θ is the angle between the vectors from the X-ray source to the observer and from the X-ray source to the centre of the primary star. σ_T is the Thompson cross section (6.65×10^{-25} cm²) and x is related to r by the equation

$$r^2 = a^2 + x^2 - 2ax \cos \theta \quad (4)$$

where a is the distance between the X-ray source and the primary star.

From equations (2), (3) and (4)

$$\tau(\theta) = (K\sigma_T/a \sin \theta) (\pi - \theta) \quad (5)$$

Since the occultation of the X-ray source starts at $\theta \sim 58^\circ$ I assume that $\tau(58^\circ) > 1$. I assume further that at phase 0.5, $\tau \lesssim 1$. Then, from equation (5)

$$1 \gtrsim \frac{K\sigma_T}{a} > 0.4 \quad (6)$$

Now if $M_x \sim 0.8 M_\odot$ for the neutron star mass, from equation (1) $M_s \sim 12.6 M_\odot$.

Then, from Kepler's third law

$$G(M_s + M_x)/a^3 = 4\pi^2/P^2 \quad (7)$$

and with the values obtained above:

$$a \sim 1.6 \times 10^{12} \text{ cm.} \quad (8)$$

Therefore, from equations (6) and (8)

$$2.4 \times 10^{36} \gtrsim K > 9.6 \times 10^{35} \text{ cm}^{-1} \quad (9)$$

Since the limits above do not differ by more than a factor three I assume

$$K \sim 2 \times 10^{36} \text{ cm}^{-1} \quad (10)$$

to obtain order of magnitude estimates.

From equation (2) the density of the wind at the neutron star orbit, $n(a)$ can be derived. Using the figures given by equations (8) and (10)

$$n(a) \sim 8 \times 10^{11} \text{ cm}^{-3} \quad (11)$$

To obtain an X-ray luminosity L_x of about 5×10^{36} erg s⁻¹ requires an accretion rate of about $7 \times 10^{-10} M_\odot \text{ yr}^{-1}$. This figure is obtained from the relation

$$L_x \approx (GM_x/R_x) (dM_x/dt) \quad (12)$$

where I have assumed $R_x \approx 10^6$ cm.

Since the neutron star moves supersonically with respect the wind, the accretion rate is given by

$$dM_x/dt = (4\pi(GM_x)^2/V_R^3) n(a) m_H \quad (13)$$

where V_R is the relative velocity between the neutron star and the wind and m_H is the proton mass. From equation (13) and the values calculated before

$$V_R \sim 1,640 \text{ km s}^{-1} \quad (14)$$

Since

$$V_R^2 = V_0^2 + V^2 \quad (15)$$

where $V^2 \sim GM_s/a$ is the neutron star orbital velocity, the wind velocity V is

$$V \sim 1,610 \text{ km s}^{-1} \quad (16)$$

On the other hand, the rate of loss of mass dM_s/dt from the primary star due to the wind is

$$dM_s/dt = 4\pi K V m_H \quad (17)$$

and, with the parameters derived above,

$$dM_s/dt \sim 10^{-4} M_\odot \text{ yr}^{-1} \quad (18)$$

The calculated wind velocity and mass loss rate are in good agreement with observational measurements carried out by Morton¹¹ and Carruthers¹² from the spectra of hot stars, which give a certain credibility to the model.

The data seem to indicate the presence of a secondary minimum at phase 0.5. This can be explained, in principle, in the framework of my model, since the matter is accreted mainly after passing the tail shock¹³, increasing locally the optical depth and reducing the X-ray intensity observations are made through the accretion cone near phase 0.5.

If the total angular momentum carried out by the wind is zero, then there should be an increase in the orbital period of the order of 5 s yr^{-1} because of the mass loss calculated above, a result which is very important from the observational point of view. (Aldo Treves called my attention to this point.)

Optical observations (UBV photometry) of this system have been made since March 1973 in São Jose dos Campos (São Paulo). The results, with a more detailed analysis of this system, will be presented soon.

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Astronomical demonstration of an infrared upconverter

AN upconverter based on a laboratory system¹ developed at the Optical Sciences Center (OSC), University of Arizona, has been used to convert infrared photons from astronomical sources to higher frequency quanta that were detected with a pulse-counting photomultiplier photometer. These observations, made at the Coudé focus of the Steward Observatory 90-inch (229-cm) reflector on Kitt Peak, represent the first successful astronomical demonstration of infrared upconversion, although infrared laser heterodyne radiometry has recently been used to detect the Sun² and α Boo³.

Upconversion is accomplished in an optically nonlinear medium, which in our upconverter is a lithium iodate crystal 5 cm long. Essentially 100% quantum conversion efficiency (QE) was achieved using a 0.6943- μm high peak power ruby laser pump developed at the OSC. This laser seems to be

unique among published systems for its efficient generation of TEM₀₀-mode radiation. Details of its design can be found in ref. 4. A 1.064- μm Nd:YAG pumped version of the upconverter yielded a QE of 0.1% (ref. 5). The Nd-YAG pumped upconverter was selected for the astronomical test because it has an infrared spectral bandwidth greater by a factor of 700 and because the effective duty cycle of the Nd-YAG laser is 65 times greater than that of the ruby laser. Therefore, in spite of its lower QE, the Nd-YAG-pumped upconverter is more efficient by a factor of 45 for the detection of thermal astronomical sources.

The astronomical infrared signal at frequency ν_s is phase matched with the pump radiation at frequency ν_p by adjusting the angle of the crystal. The upconverted signal, $\nu_u = \nu_p + \nu_s$, is detected by a pulse counting photometer using a cooled gallium arsenide photomultiplier (RCA C31034). The astronomical source is acquired at the Coudé focus of the 90-inch reflector and directed into the upconverter entrance aperture. The infrared radiation is focused at the appropriate position within the nonlinear crystal. Guiding is accomplished by viewing the image of the source reflected by a dichroic beam splitter to a reticle eyepiece.

Because of the extreme sensitivity of phase matching to angular perturbations, the alignment of the telescope optical axis with the laser pump beam in the nonlinear crystal is extremely critical. We have found it necessary to devote considerable time to this task, and to install auxiliary HeNe alignment lasers to monitor the orientation of optical elements in the system.

The instrument simultaneously upconverts radiation between 3.2 μm and 5.0 μm . This spectral bandwidth of 1.8 μm is the largest ever reported for an upconverter operating in this spectral region⁵. The central wavelength of the operating bandwidth was selected by rotating the lithium iodate crystal about a perpendicular to its optical axis.

During the observations, the laser was pulsed at rates ranging from 0.5 to 4 s^{-1} with a stable pulse width of 2.0 ms and a typical pulse energy of 0.3 J. The Nd:YAG laser is capable of operating at 30 s^{-1} . But we usually operated at 0.5 s^{-1} to permit real-time statistical analysis of the data using an HP-45 portable calculator. Raw data also were printed on paper tape.

The observations were made on the nights of September 8 to September 14, 1973. We attempted to detect the Moon, Mars, α Aur, α Tau, α Ori, Venus, α CMa, ρ Per, NGC1068, α Her, and β Peg. Integrations taken on each object were followed by integrations on the adjacent blank sky. This less-than-optimum detection procedure was used because the telescope was not equipped with a wobbling secondary mirror. The statistical detections are relative to the adjacent sky. An ice dark slide and a glowbar resistor source were also observed. The glowbar data were used to monitor system stability. The ice dark slide data indicated that in practice the thermal background of the detector was definitely less than that due to the telescope and sky.

Multiple statistically significant detections of the Moon, α Tau, α Ori, Venus and α Her were accomplished. Typical data from individual observations with integration times of 0.1 and 0.2 s on September 10, 1973 are summarised in Table 1. These integration times were adequate to yield 2σ (standard deviation) detections of the first four listed sources. Combining data from multiple observations of this type, higher confidence results are obtained.

Table 1 Typical observational data

Object	Signal-to-noise ratio	Integration time* (s)
Moon	2.4	0.10
α Ori	2.2	0.20
α Tau	1.6	0.20
Venus	2.8	0.10
α CMa	0.4	0.20

*Laser pulse of duration 2.0 ms at 0.5 pulse s^{-1} .

Tests showed that a system quantum efficiency (SQE) of approximately 0.001% was attained. This value includes quantum efficiency losses in filters and in the photomultiplier tube. To increase SQE, improvement in the quality of lithium iodate crystals is required, because the currently available material suffers from an absorption which is 0.5 μm wide and centred at 4.3 μm . This absorption results in excess thermal noise from the upconverter when operated at room temperature. We used dielectric interference filters to remove stray flashlamp light from the laser pump beam. The present state of the art should allow better filters, with high transmissivity at 1.064 μm and very low transmissivity at wavelengths below 0.95 μm , to be made. With improvements in the crystal and filter quality, a theoretical SQE of approximately 1.0% could be attained by an upconverter of this type.

We plan to test the upconverter as a device for infrared spectroscopy. This is possible because the upconversion process preserves the frequency coding of the input infrared signal. It will be possible to detect the entire upconverted spectrum simultaneously with a photoemissive image tube because the infrared spectrum is shifted to the 0.83- μm region.

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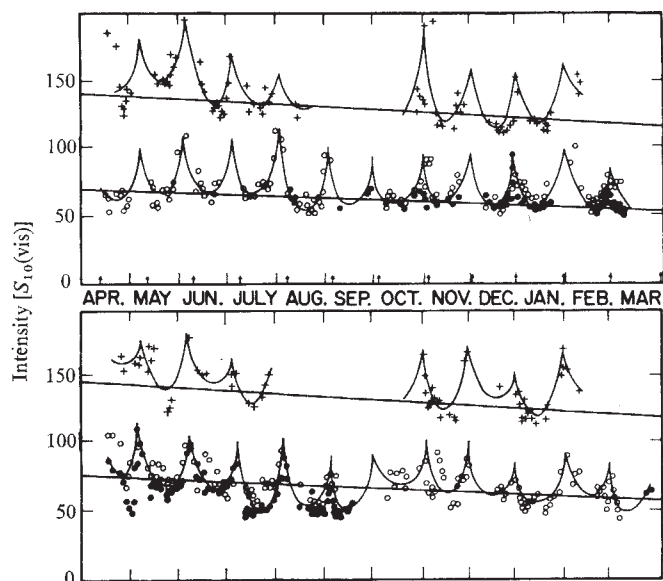


Fig. 1 Zodiacal light data of Levasseur and Blamont (Fig. 4 of ref. 4). Mean value of the intensity for eight directions: top graph $\epsilon < 0^\circ$; +, ecliptic plane; O, $+45^\circ$; ●, south pole. Bottom graph $\epsilon > 0^\circ$; +, ecliptic plane; O, $\pm 45^\circ$; ●, north pole.

Measurements were then made at $\epsilon = 90^\circ$ and varying ecliptic latitudes, usually with $\beta = \pm 35^\circ$. Readings were taken at the rate of three a second for 15 min of each 96-min orbit with a night-time look height of 400 km or higher. There were 15 orbits a day for most of four years. Usually, 1,880 readings or 5 min of data were reduced to determine the brightness and polarisation for that night. So detection of changes down to a time scale of hours was possible through the four year period with the following exceptions.

The previously published results^{2,3} did not include readings when: (1) the Moon was within 50° of the field of view (one week out of four); (2) a first magnitude object was on the edge of the field of view (seldom); (3) the galactic latitude was less than 30° (four out of 12 New Moon periods). Given these exclusions an absolute calibration of 10% over four years was made. The relative calibration precision over any New Moon period was found to be 5% or less.

The effect on the Moon on telescope 3 was most evident during either first or third quarter depending on the orientation of the spin axis. At these times the Moon could be within 50° of the spin axis. The airglow telescopes in the opposite sail direction were severely hampered any time the Moon was above the satellite horizon. This was because protruding parts of the spacecraft at times allowed reflected moonlight to fall within 50° of the spin axis. (Nightglow measurements were not made with the Moon up.)

The six-parameter least-squares fit made in reducing a 5-min data interval proved of great value in detecting contamination light scattering into the optics from the Moon, moonlight reflected from the Earth, and bright stars and planets near the field. In such cases a considerable variation of intensity at the spin rate as well as at the polaroid frequency, which was twice the spin rate, was seen. Without such a check it would have been more difficult to avoid periodic variations with a lunar period in the data.

So the effects of the Moon may be appreciable any time it is above satellite horizon. The time of maximum effect depends on the particular geometry involved. We have

Is the zodiacal light intensity steady?

CONTINUED refinement of data on zodiacal light from OSO-5 (ref. 1) confirms our conclusion^{2,3} that the absolute value of both the surface brightness and polarisation of the zodiacal cloud varied by less than 10% over the 4-yr period from January 1969 to January 1973. These results are in stark contrast with the recently published satellite measurements of Levasseur and Blamont⁴.

The blue photometer, one of six, which recorded the bulk of the zodiacal light measurements comprised a broadband system with an effective wavelength of 4,180 Å, an 11° circular field of view and a fixed polaroid. The photometer viewed along the anti-sail spin axis of the satellite which rotated once every 2 s. The spin axis was held in a plane whose normal was directed at the Sun. Its movement within this plane varied from 0.1° to 1.0° d^{-1} .

placed a periodic variation typical of reflections, with lunar frequency, over the data of Levasseur and Blamont (Fig. 1). No attempt at an exact fit to the data has been made, and much of the Full Moon data had already been removed. Nevertheless the chief peaks in the data of Levasseur and Blamont fall between first quarter and Full Moon, except for the initial peak which comes within a few days of launch. Further the New Moon data are quite free of the fluctuations otherwise seen, and a straight line drawn through their New Moon data has a consistent slope in all four sets of data indicating photometer degradation of some $20s_{10}[\text{vis}]$ per year in excess of the calibrations that were used to reduce the data.

Data with galactic latitude less than $\pm 30^\circ$ have also now been studied. For data closer to the galactic plane, the absolute calibration errors increased from 10% to 30% due to increased problems of star background and diffuse galactic light subtraction. The relative calibration precision over any New Moon period increased from 5% to 10%. Within these limits again no variations were found in either the brightness or polarisation of the zodiacal light.

A relative variation of 10% or less was observed during the periods April 15 to 29, 1971, and November 2 to 16, 1971. These results are in direct conflict with the satellite measurements of Levasseur and Blamont⁴ who have recently reported enhancements of 50% on April 21, 1971, with a half enhancement lasting 4 d, and a 100% enhancement on November 7, 1971, with a half enhancement of 6 d. The enhancements were attributed to debris of comets 1961-I and Biela.

Figure 2 shows our results for surface brightness and polarisation during these times. The absolute intensities during the April period are higher than the November period because the ecliptic latitude is lower. The scatter in the data is caused by the difficulties in subtracting out the star background near the galactic plane. It is typical of our relative calibration precision of 10% at low galactic latitudes. From April 15 to 29, 1971, our measurements were taken at $\epsilon = 90^\circ$, $\beta = 10^\circ \pm 2^\circ$. For comparison, the results of Levasseur and Blamont report an increase of 50% at $\epsilon = 90^\circ$, $\beta = 0^\circ$, a 50% increase at $\epsilon = 90^\circ$, $\beta = 90^\circ$, and a 50% increase at $\epsilon = 90^\circ$, $\beta = 45^\circ$. From

November 2 to 16, 1971, our measurements were taken at $\epsilon = 90^\circ$, $\beta = 30^\circ \pm 2^\circ$. During this period Levasseur and Blamont report an increase of 100% at $\epsilon = 90^\circ$, $\beta = 0^\circ - 30^\circ$, and no increase in the $\epsilon = 90^\circ$, $\beta = 90^\circ$, directions. In addition, none of the other sizable variations prevalent in the data of Levasseur and Blamont from April 1971 until March 1972 have been found in our data covering this period.

On two occasions, the Lagrangian points L4 and L5 of the Earth-Moon system passed within 2° of the centre of the field of view of our blue zodiacal light telescope. These events took place on January 29, February 7, 1971, and June 25, July 6, 1971. No change in either the intensity or polarisation to the limiting accuracy of the experiment (10%) were observed. This sets a full field upper limit of $10s_{10}(\text{blue})$ for any change in surface brightness and $2s_{10}(\text{blue})$ for any change in polarisation due to scattering from dust accrued at these points.

We believe the zodiacal cloud to be a smooth, regular feature of the Solar System as one might expect from a steady influx of particles from the continual breakup taking place in the asteroid belt. There is no evidence for a cloud, with intensity fluctuations of a factor of two, composed of an interwoven maze of comet trails. No variations in intensity or polarisation greater than the limit of accuracy of the experiment—usually 10% arising from comets, solar activity, lunar phase or other events in the Solar System—were observed.

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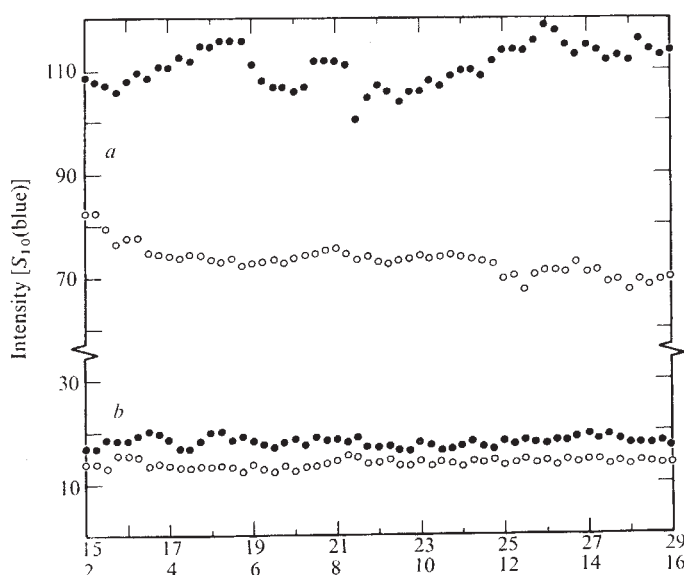


Fig. 2 The brightness (a) and polarised intensity (b) of the zodiacal light as measured by OSO-V; ●, April 15 to 29, 1971; $\epsilon = 90^\circ$; $\beta = 10^\circ \pm 2^\circ$. ○, November 2 to 16, 1971; $\epsilon = 90^\circ$; $\beta = 30^\circ \pm 2^\circ$. Absolute calibration precision = 30%, relative calibration precision = 10%.

f Gravity and gravitational singularities

TRAUTMAN has postulated¹ that the usual space-time singularity occurring in classical cosmological models and in the gravitational collapse of massive objects could be averted if intrinsic spin effects are incorporated into general relativity by adding torsion terms to the usual Einstein field equations, that is through the Einstein-Cartan theory. Invoking a primordial magnetic field for aligning all the individual nuclear spins he shows that his universe consisting of 10^{80} aligned neutrons collapses to a minimum radius of the order of 1 cm with a corresponding matter density of $10^{55} \text{ g cm}^{-3}$.

Salam *et al.*² have investigated the effect of taking *f* gravity into account in Trautman's model. They consequently obtain profound changes in the numerical values of the parameters, the minimum radius of the Trautman universe now being of the order of 10^{13} cm rather than 1 cm, implying that spin-aligned hadronic matter will not collapse to densities higher than $10^{17} \text{ g cm}^{-3}$ —only two or three orders of magnitude higher than that of nuclear matter. But the perfect spin alignment necessary for these results still remains difficult to realise. We now suggest a possible way to avoid this difficulty and still retain all the results.

Two of us have shown³ that perhaps the easiest and most natural way to include the short range of *f* gravity (mediated by massive spin 2^+ *f* mesons) into Einstein's field equations was to reinterpret the so-called 'cosmological' constant Λ in

terms of the inverse Compton length of the f meson

$$\Lambda \simeq \left(\frac{m_f c}{\hbar} \right)^2 \quad (1)$$

m_f being the mass of the f meson.

In the massless (infinite range) gravity field of conventional general relativity mediated by massless gravitons, $\Lambda=0$.

Now the usual Friedmann equation with Λ , for a zero pressure Einstein-de Sitter universe is

$$\dot{R}^2 = \left(\frac{8}{3} \right) \pi G \rho R^2 - \left(\frac{2}{3} \right) \Lambda c^2 R^2 \quad (2)$$

with $M = \left(\frac{4}{3} \right) \pi R^3 \rho$, being the total mass of the 10^{80} baryons in the Universe. Using $R=0$ at $t=0$, gives

$$R(0) = \left(\frac{3GM}{\Lambda c^2} \right)^{1/3} \quad (3)$$

Λ is defined as in equation (1), where $m_f \simeq 1,500$ MeV. Substitution in equation (3) yields $R(0) \simeq 1$ cm and the corresponding density of matter is $\rho = \left(\frac{\Lambda c^2}{4G} \right) \simeq 10^{55}$ g cm $^{-3}$. These

values are the same as in Trautman's model. It turns out that the Λ term in equation (2) is of the same order of magnitude as the torsion term in Trautman's model. In both cases these additional terms (torsion term in Trautman's, Λ term in ours) in the Friedmann equation are the ones which avert the singularity. For $\Lambda=0$ (the massless gravity field of infinite range), we recover the old singularity. If we invoke f gravity (and especially as matter in a superdense collapsed state is predominantly composed of hadrons it is reasonable to consider the gravitational interaction between them at short range to be due to exchange of massive f mesons), we must necessarily use the corresponding constant G_f . Therefore, replacing the Newtonian constant G by G_f , the value of which has been estimated⁸ to be of the order of $10^{39}G$, we obtain $R(0) \simeq 10^{13}$ cm and the corresponding matter density as $\simeq 10^{17}$ g cm $^{-3}$. These results are the same as obtained by Salam *et al.* We note in particular that in our formulation the difficult hurdle of finding a suitable mechanism for the alignment of all hadron spins is not encountered. We can understand this result by noting that the Hawking-Penrose⁴ singularity theorems for the Einstein field equations $G_{\mu\nu} + \Lambda g_{\mu\nu} = \kappa T_{\mu\nu}$, do not necessarily hold if the cosmological constant Λ is positive, which is implied by our definition in equation (1). Indeed it is remarked in ref. 2 that for all spins aligned in the same direction, the effective spin-spin interaction term in the Lagrangian for the Einstein-Cartan theory which forms the essence of Trautman's torsion term, can be regarded as being of the form $L = -(-g)^{1/2} \Lambda_{eff}(x)$, where $\Lambda_{eff}(x)$ is a positive effective cosmological contribution to the Lagrangian. Moreover, as we pointed out earlier, the Λ term we have used in the Friedmann equation is of the same magnitude as Trautman's torsion term.

It is interesting to note that by using G_f instead of G , the absurdly large density $c^5/G^2\hbar \simeq 10^{94}$ g cm $^{-3}$ at which quantum effects of the gravitational field are expected to become important is scaled down to $\simeq 10^{17}$ g cm $^{-3}$. The corresponding Planck length $(\hbar G/c^3)^{1/2} \simeq 10^{-33}$ cm, at which quantum fluctuations in the space-time curvature are supposed to become significant⁵⁻⁷, is then scaled up to $\simeq 10^{-14}$ cm. This probably indicates that the quantum effects in f gravity become important during much earlier stages in the collapse, when the density is $\simeq 10^{17}$ g cm $^{-3}$.

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Trends in the vertical distribution of ozone over Australia

KOMHYR *et al.*¹ reported predominantly positive trends in total ozone content over several stations in the 1960s (see also refs 2-4). Here I report the first detailed estimates of trends in the vertical distribution of ozone and discuss some possible causes.

Soundings of the vertical distribution of ozone have been made on a regular weekly basis at Aspendale, Australia (38.0°S, 145.1°E) since June 1965 (ref. 5). Some 432 soundings reached above the 18.1 mbar (27 km) level during the first 8 yr of the programme. Data from these soundings have been corrected to agree with the total amount in a vertical column measured with a Dobson spectrophotometer, by the method⁶ in which the ozone concentration above balloon burst is extrapolated along a line of constant mixing ratio for burst heights above the 18.1 mbar level. Extrapolated data are used to calculate the correction factors, but are excluded from the mean data used in trend analysis.

Estimated trends are based on a least squares linear regression against time of 48 successive deviations of 2-month mean ozone partial pressures (from the 8-yr means for the corresponding months) for each of 36 standard levels from 1,000 mbar to 10 mbar. The results are shown in Fig. 1.

Outstanding features are the statistically significant decreases in ozone content—of the free troposphere (chance probability $P < 1\%$ between the 700 and 450 mbar levels), and around the level of the primary ozone maximum ($P < 0.1\%$ at 35 mbar)—and significant increases in ozone content above the 17 mbar level ($P < 0.1\%$ at 12 mbar).

Over the same period of 8 yr the total ozone content as measured by the Dobson spectrophotometer at Aspendale shows an estimated trend of $(-3.1 \pm 1.1)\%$ per decade.

Table 1 shows these estimated trends in the total ozone content, in the integrated ozone amounts in 10 km thick layers up to 30 km, and in the integrated amount above 30 km (the difference in the trends in the total amount and in the amount below 30 km), all in units of thickness of ozone (reduced to STP) per decade.

Table 1 Ozone trend estimates in various layers of the atmosphere in units of reduced thickness of ozone per decade (data June 1965 to May 1973 inclusive)

Layer (km)	Trend (10 ⁻³ atm cm per decade)
0 - ∞	-9.9
0 - 10	-2.7
10 - 20	-6.7
20 - 30	-11.2
Above 30	+10.7

The mean total ozone content during the 8-yr period is 0.318 atm cm, and the integrated amount above 30 km is 0.058 atm cm. Thus the mean increase above the 30 km

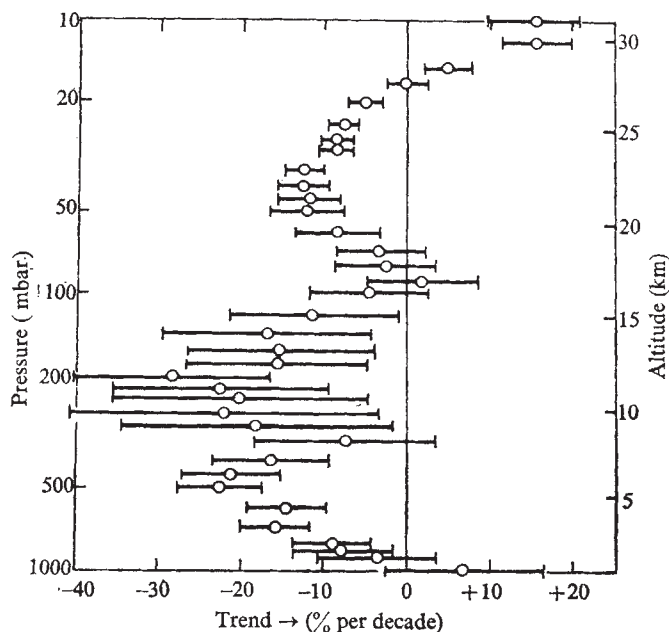


Fig. 1 Estimated trend (% per decade) of ozone partial pressures at 36 standard levels over Aspendale (38°S, 145°E) for 1965-73. Standard errors of the estimates are indicated.

level is 18.5% per decade, in good agreement with the direct trend estimate of $(+15.3 \pm 5.6)\%$ per decade at the 10 mbar (31.1 km) level (Fig. 1).

Trends in the vertical distribution of ozone of such magnitude and statistical significance are of interest in relation to questions of climatic change, global monitoring and pollution, and ozone photochemistry and interacting circulation processes.

Mean annual cycles of ozone partial pressure (nbar) at the 10 mbar and 40 mbar levels are shown in Fig. 2 for Aspendale, with comparable data for Thalwil, Switzerland⁶ (47°N) and Boulder/Albuquerque^{6,7} (37½°N). As well as the interhemispheric comparison (which will be discussed elsewhere), these curves confirm that the ozone concentration at the 10 mbar level is dominated by photochemistry (maxima in summer) whereas that at 40 mbar is dominated by transport processes (maxima in the late winter and spring).

One might reasonably suggest that the trends at and above the 10 mbar level are due to some process affecting the ozone photochemistry, such as some solar influence or a circulation-induced change in the photochemical process such as a change in the upward flux of NO_x . The trends below the 17 mbar level are most plausibly related to a change in circulation which either reduces the downward flux of ozone from the region of photochemical production or else increases the concentration or flux of some chemical species which destroys ozone in the lower layers. Perhaps the simplest hypothesis is to postulate increased static or dynamic stability at about the 17 mbar level.

Trend analyses of the deviations of 96 consecutive monthly mean temperatures from the 8-yr means for the corresponding months at Laverton, Victoria (the nearest aerological station, some 35 km north-west from Aspendale) show no statistically significant trends at the 10, 15, 50, 100 or 200 mbar levels. Similarly there are no significant trends in the temperature gradients between the 10 and 15, 15 and 50, 50 and 100 or 100 and 200 mbar levels which might have indicated a change in static stability. Estimation of possible trends in the dynamic stability is beyond the scope of the present work, but I note that the available wind data became increasingly inadequate above the 50 mbar level. Any such trend has not resulted in a significant change in the mean temperature or temperature gradient

at any of the levels listed above.

Trend analyses of two parameters of the general circulation, the mean latitude of the high pressure belt⁸ and the abbreviated southern oscillation index⁹, indicate such small trends that they are clearly not related to the observed trends in ozone.

Long term trends in the ozone content above the 30 km level have been tentatively reported¹⁰ and related to the solar cycle. Paetzold *et al.*¹¹ reported a significant positive correlation between ozone content in the region 20-30 km and sunspot number. They suggest a direct causal link with variations in solar radiation at about 210 μm with no significant phase lag. But the Aspendale data, which span the sunspot maximum of 1968-69, show no evidence of a change in the sign of the trend.

The observed decrease in ozone content below the 17 mbar level could be due to *in situ* destruction by contaminants such as volcanic dust or NO produced by nuclear bomb tests¹². There is, however, little to support this from the ozone record following the Mt Agung eruption in 1963 (refs 3 and 13) or the resumption of atmospheric bomb testing in 1962-63 (ref. 3), unless the time scale for such effects is so long that no statistically significant departures from normal were to be expected in 1963 or 1964 (ref. 14).

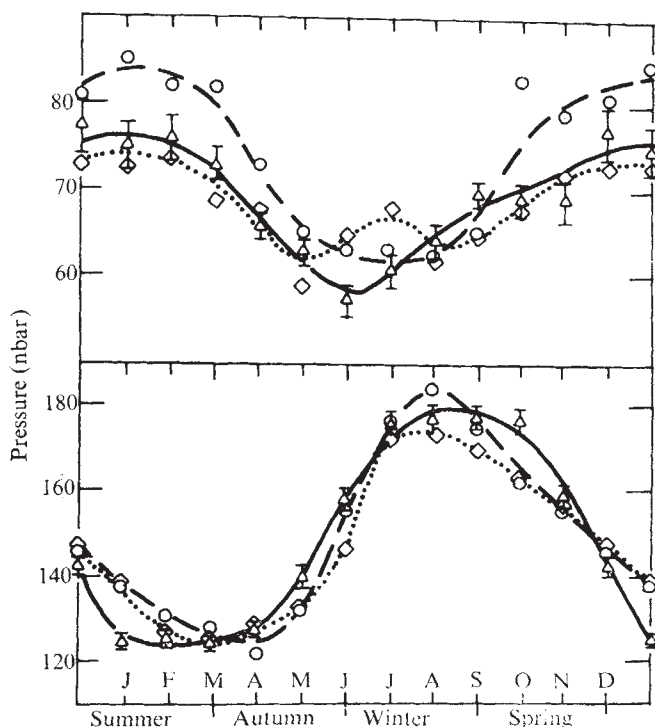


Fig. 2 Mean annual cycle of ozone partial pressures (nbar) at the 10 mbar level (upper diagram) and at the 40 mbar level (lower diagram) for: Δ , Aspendale (38°S); $\circ$, Boulder/Albuquerque (37½°N); $\diamond$, Thalwil (47°N). Standard errors are shown for the Aspendale results.

I conclude that the cause of the observed ozone trends is at present an open question. These trends are large enough to warrant careful attention in relation to questions of climatic change, global monitoring and ozone photochemistry. More data are needed from more stations and over longer time intervals, and the various possible explanations must be exhaustively tested by all available means.

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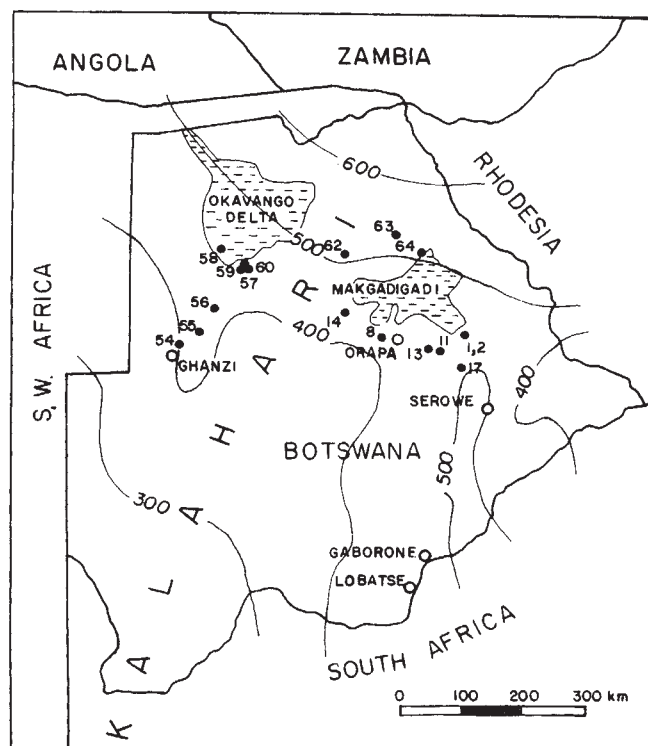


Fig. 1. Sample locations referred to in Table 1.

Radiocarbon and tritium evidence for direct rain recharge to ground waters in the northern Kalahari

THE Kalahari, a flat and sand-covered region of more than 600,000 km² (Fig. 1), has long been classified as a desert because of the virtual absence of surface waters in the form of springs or perennial rivers. The only exception is the inland drainage system in the north, the Okavango delta and Makgadigadi salt pan, which are fed successively by rainwater which drains from the remote highlands of Angola through the Okavango river.

Many investigators¹⁻⁶ have subscribed to the view that a sand over-burden exceeding an estimated 6 m would effectively prevent infiltrating rainwater from reaching phreatic water tables. All rainwater retained in the sand would thus be lost by capillary rise and evapotranspiration, chiefly during the dry season. In the area under study, with varying sand cover thickness, the possibility of direct rain recharge was consequently ignored in economic surveys and the ground waters considered to be fossil relics from earlier, much wetter periods.

Table 1 ¹⁴C and tritium in phreatic ground water of the northern sand-covered Kalahari

No.	Well	¹⁴ C (% m.c.)	Tritium (TU)
2	Makgaba 2	120.2±2.6	4.3±0.3
14	Rakops	106.1±4.5	24.4±1.7
54	Khodabis 1231	99.1±2.2	5.1±0.6
60	Toteng 1242	98.7±1.8	16.9±1.7
55	Z 1183	97.7±2.1	15.8±1.3
63	Gweta	97.6±1.5	1.2±0.3
62	Bushman Pits	97.4±2.2	8.0±1.0
1	Makgaba 1	93.7±1.7	5.0±0.5
64	Zoroya 1606	91.9±2.2	10.3±1.1
8	Steinberg's BH	86.2±1.6	2.8±0.4
17	Tshepe	78.4±1.3	1.6±1.6
11	Makoba	78.0±1.4	—
13	Mahata	69.1±1.3	0.7±0.3
58	Tsau	68.3±1.3	0.9±0.2
56	Kuke	66.8±1.7	0.6±0.6
59	Toteng 1320	59.5±1.0	—
57	Sehitwa tribal borehole	54.3±1.2	1.2±0.3

Recent investigations we have carried out in collaboration with the Geological Survey of Botswana have shown that rain recharge does occur in areas covered with sand. A controlling factor is the annual amount of precipitation, which averages 450 mm in the north, gradually decreasing to 250 mm in the south. In addition, the seasonal variability of annual rainfall with the occasional high rainfall outliers (such as the seasons of 1966–67 and 1973–74) is of extreme importance for recharge.

We sampled several phreatic wells in the northern Kalahari and the environmental ¹⁴C ($t_{1/2}=5,730$ yr) and tritium ($t_{1/2}=12.3$ yr).

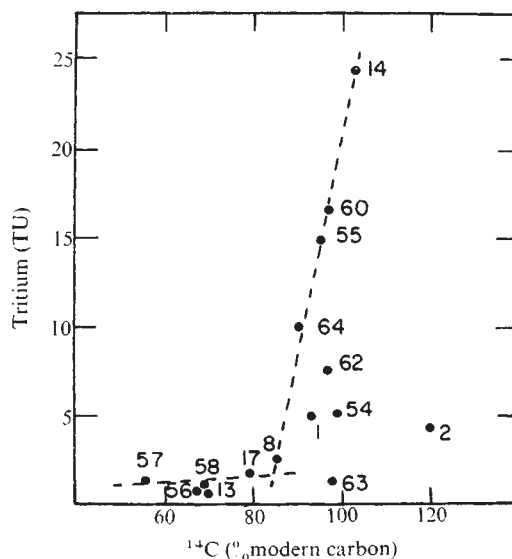


Fig. 2. Radiocarbon-tritium correlation. Samples with values greater than 85% modern carbon radiocarbon and greater than 2 TU of tritium contain a post-nuclear bomb component. Dashed lines, theoretical trends for pre-bomb and post-bomb waters.

12.3 yr) data are reported here (Table 1 and Fig. 1).

Since 1952 nuclear bomb tests have increased the radiocarbon content of atmospheric carbon dioxide above the pre-bomb level of 100% modern carbon (m.c.). Levels in the Southern Hemisphere reached a peak of 165% m.c. in 1963 and decreased to 145% m.c. in 1972 (ref. 7). Biogenic carbon dioxide is dissolved in infiltrating ground water and then reacts with lime free of ^{14}C to produce dissolved bicarbonate. Carbon dioxide at 100% m.c. will produce bicarbonate containing 60 to 90% m.c. through this dilution, with an average of about 85% m.c. (ref. 8). All values above 85% m.c. in Table 1 can therefore be regarded as containing post-bomb recharge, most clearly indicated in the first two cases at 106 and 120% m.c.

The average annual value of tritium in continental rains before the inception of bomb tests in 1952 is estimated at around 5 TU (tritium units). Southern African values reached 60 TU in 1963 and have decreased to about 25 TU at present (ref. 9 and unpublished data). Undiluted recharge at 5 TU in 1952 has decayed to less than 2 TU at present. Therefore, all waters with more than 2 TU must contain a component of recharge from the last 20 yr.

The observations (Table 1 and Fig. 2) corroborate the basic assumptions in the analysis of the tritium and ^{14}C results. The two lines, which tend to define the limits of the results in the pre-bomb and post-bomb eras, intersect at about 2 TU and 85% m.c. The low tritium concentration values to the right of the limit line may indicate different responses to atmospheric bomb tritium and ^{14}C increases. The shallow (1 m) sample no. 2 might present a special case in that roots tapping the water enhance downward transport of ^{14}C .

Ages greater than 20 yr are therefore indicated by values below 85% m.c. The lack of secure theoretical foundations does not permit accurate ^{14}C age calculations. But with an initial value of 85% m.c. waters with 50% m.c. would still be more recent than one ^{14}C half life. Waters in the range of 50 to 85% m.c. may therefore be regarded as having ages from a few hundred to a few thousand years at most, which implies that they are hydrologically active.

Both the radiocarbon and tritium data lead to the unambiguous conclusion that ground waters in the northern Kalahari are directly, and in some cases rapidly, recharged by rain.

Balanced exploitation could make room for additional recharge which is partly lost to evapotranspiration from the often shallow water tables. Even at values of rainwater infiltration as low as 5%, this could still constitute a significant managed ground water supply.

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Geomorphology of Siluro-Devonian alluvial plains

THE Anglo-Welsh outcrop of the Lower Old Red Sandstone (Siluro-Devonian) reaches approximately 2,000 m in thickness and is largely alluvial¹, consisting of long sequences of fining upwards cyclothems². The fine grade (floodplain) members of the cyclothems commonly include one or more pedogenic carbonate units which are evident in the vertical profile³⁻⁶. The carbonates seem to represent episodes of soil formation, each of which lasted for about 10^4 yr. Well horizonated palaeosols indicate periods of prolonged weathering, which implies that the alluvial plains on which they were formed remained unflooded for long periods. This could have arisen either because rivers shifted about on the plains, or because of a cyclical river dissection-aggradation coupled with lateral movements. The distribution of Quaternary calcretes⁷ suggests that the climate was hot (mean annual temperature 16-20° C) with a low seasonal rainfall (mean annual precipitation 100-500 mm). This is supported by palaeomagnetic results, which indicate a low (southerly) latitude for the Anglo-Welsh area⁸.

Four plausible models are considered, which give sequences consistent with known river behaviour and which are applicable to alluvial sequences in the Lower Old Red Sandstone and similar formations.

Model A. Each river, by step-like channel movements combs systematically from side to side of an alluvial cone, in the manner of the Kosi⁹ and Indus¹⁰⁻¹¹. Soil formation near one side is most intense when the river is active at the opposite side. The resulting palaeosols are discrete, laterally extensive, but non-uniform sheets, each equivalent in time to numerous channel sand-bodies (coarse grade cyclothem members). Provided that the distance moved by the active channel at each step exceeds the width of the channel belt (if braided) or meander belt (if sinuous), vertical profiles show cyclothems with a range of numbers of palaeosols. Fine grade members with no palaeosols result from the erosional emplacement of channel deposits. Control in this model is autocyclic¹², that is, intrinsic to the fluvial regime, and external factors such as climatic change, eustasy and tectonism need not be involved.

Model B. Rivers periodically shift major lengths of their channels by large, almost random distances. This is described as avulsion. It also has an autocyclic control and is well known from modern streams^{13,14}. Because the effective zone of the river is laterally restricted, soils can form at sites more distant from the stream. The repeated avulsion gives an alluvial sequence in which palaeosols repeatedly split around bodies of floodplain sediment. The pedogenic carbonates in this model are partly interconnected, either fusing with other palaeosols or having erosional or feather-edge margins. Provided that the lateral distance moved by a river at each avulsion substantially exceeds the width of the channel belt or meander belt, vertical profiles through the alluvium will show cyclothems with contrasted numbers of palaeosols. Each palaeosol is the time equivalent of a number of coarse grade members formed elsewhere. Erosional emplacement of coarse grade members partly explains cyclothems which have no palaeosols. Some floodplain sequences, however, may never have undergone pedogenesis.

Model C. Cyclical river dissection-aggradation occurs because of climatic fluctuations between more and less arid conditions. Following inferred events in the Australian Riverine Plain^{15,16}, the Lake Torrens Piedmont¹⁷, the Algerian Aurès Mountains¹⁸, and the valley of the Rio Grande¹⁹⁻²², a shift into relative aridity causes a reduction in the discharge of the river, which leads to channel dissection. The diminished stream lies entrenched in a shallow, possibly terraced, valley, and pedogenesis begins on the terraces and the interfluvial plains, which are no longer flooded by the river. With a climatic amelioration, river discharge again increases and valley filling begins. Soil formation is halted, first on the terraces, and then on the interfluvial plains, as floods become more frequent and widespread.

As soon as the valleys are fully aggraded, the rivers can move laterally by avulsion until the next climatic reversal, by which time they will be in new positions. The alluvial piles thus created have discrete pedogenic carbonates of fairly uniform lateral development, largely embedded in floodplain deposits. Provided that the rivers shift laterally by substantial amounts during each climatic amelioration, the cyclothems show a range of numbers of palaeosols in vertical profile. Fine grade members without palaeosols are largely a result of the erosional emplacement of channel deposits. The main control in this model is allocyclic¹², that is, external to the river regime, though autocyclic avulsion is at times prominent.

Model D. This model is similar to model C but uses a change of base level as the allocyclic control, in combination with avulsion. A fall of the base level causes river entrenchment and promotes soil formation on the exposed interfluvial plains, whereas a rise leads to valley aggradation and river avulsion, and stops pedogenesis in interfluvial areas. For example, rivers crossing the Gulf Coastal Plain to the Gulf of Mexico became entrenched during the Pleistocene glaciations, so that soils formed on the interfluvial plains, but during interglacial periods they aggraded their valleys²³. The cyclothems and alluvial structure of model D are similar to those of model C. In vertical profile the cyclothems may again contain more than one palaeosol. The erosive emplacement of coarse grade members explains the absence of palaeosols from some cyclothems. model D remains plausible, however, only if the change of base level is less than approximately the channel depth. With bigger ranges, the theoretical coarse grade members are inconsistent with field data, to judge from modern rivers¹⁴: they are much thicker than the 2–3 m typical of lateral deposits and coarse grade members in the Lower Old Red Sandstone⁵, and they are multistorey, with evidence of a change from a braided to a meandering channel pattern during aggradation.

With models A and B, the Siluro–Devonian alluvial plains comprise, at any instant, constructionally active and inactive zones in juxtaposition, with relief arising from alluvial ridges either occupied or recently abandoned by streams. Models C and D provide a more complex picture, though again of juxtaposed active and inactive zones. At one extreme of the controlling cycle, the plains comprise shallow valleys with active rivers and broad interfluvial areas devoid of major drainage. Relief arises from alluvial ridges, active and abandoned, and from valley sides and perhaps river terraces. At the other extreme, the plains are less diverse geomorphologically, with relief provided as in models A and B.

These models provide three alternative possible gross structures for the alluvial sequence. They differ in the three-dimensional fit of the cyclothems, and in the shape, development, and degree of interconnection of the included palaeosols. The structures are all plausible in terms of the essentially one-dimensional vertical profiles observed in the Lower Old Red Sandstone. Finer distinctions demand that an adequate number of cyclothems can be confidently traced laterally over distances of the order of kilometres. This will be possible in the Anglo–Welsh area only through extensive, and expensive, shallow borings. Nonetheless, model D may perhaps be preferred. Recent work shows that the Temeside Shales (?late Silurian), near the base of the Lower Old Red Sandstone, possess palaeosols. As these beds resemble intertidal deposits²⁴, base level fluctuations could explain the episodes of pedogenesis.

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Orogenic zones in central Australia: intraplate tectonics?

THE plate tectonics model^{1,2} has been successful in explaining the orogenic features of the ocean basins and continents associated with the dispersal of Pangaea in post-Triassic time. Dewey and Bird³ extended these concepts, suggesting that there is a unique relationship between orogenesis and plate-margin tectonism, and various authors have, accordingly, interpreted certain Precambrian and Palaeozoic orogenic zones or 'mobile belts' as plate sutures^{4,5}. We argue here that plate-margin tectonism is only one of several possible conditions for the development of an orogenic belt. We suggest that the plate tectonic model of orogenesis needs to be extended and modified, and this suggestion is discussed for Gondwanide Australia. Recent attempts to impose a rift (aulacogene)⁶ or subduction⁷ model for orogenesis in central Australia are confounded by the observed geological and geophysical facts pertinent to this region.

Three zones of major thrust faulting in crystalline basement rocks have been recognised in central Australia. They bound the Ngalia and Amadeus Basins (Fig. 1) and are characterised by steep gravity gradients⁸. The Amadeus and Ngalia Basins are intracratonic depressions containing latitudinally folded and thrustured Proterozoic and Palaeozoic sedimentary cover rocks^{9,10}. The Musgrave Block, south of the Amadeus Basin, was deformed about 600 million years (Myr) ago during the 'Petermann Ranges Orogeny'¹¹ when the Woodroffe Thrust developed in the basement south of the basin. The Arunta basement complex was deformed during the 'Alice Springs Orogeny'¹¹ in late Devonian or early Carboniferous time¹². While the basement rocks of the northern margins of the Amadeus and Ngalia Basins underwent major thrusting and retrograde metamorphism, the overlying Proterozoic cover rocks deformed independently above a decollement horizon⁹.

Thick syntectonic molasse-type sediments are associated with the Alice Springs and Petermann Ranges events. Unconformities in the cover sediments, and the thickening and coarsening of facies against the northern margin of the Amadeus Basin¹³ indicate that the crystalline basement was

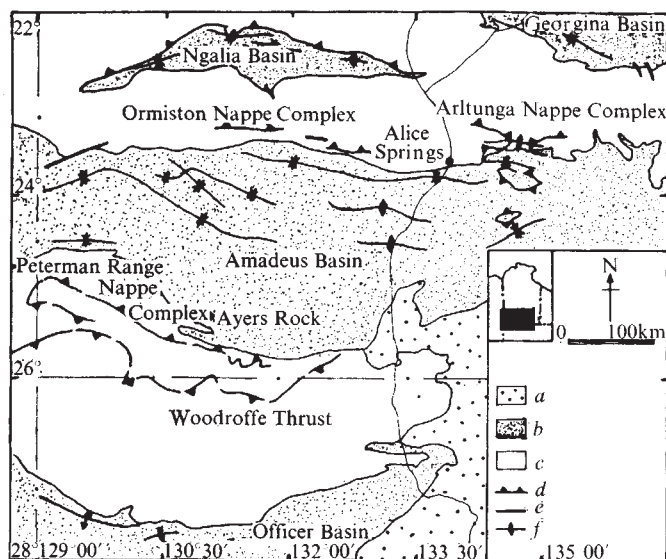


Fig. 1 The location and major structural features of the intracratonic basins and deformed basement zones of central Australia. a, Mesozoic strata; b, Proterozoic and Palaeozoic cover; c, older Precambrian basement rocks; d, thrust fault, indicating upper plate; e, fault; f, major fold.

uplifted at various times between the thrusting episodes.

Features indicating plate-margin tectonism, such as the presence of volcanics, acid intrusives, ophiolites and high pressure, low temperature metamorphics¹⁴, are absent in central Australia; only retrograde metamorphism of the amphibolites and granulites of the basement occurred during the thrusting and folding. The Giles Complex, which contains the only ultramafics in central Australia, is made up of isolated intrusions¹⁵. The distribution of the various deformed zones and the widespread, relatively undeformed nature of the late Proterozoic and Palaeozoic platform sediments preclude any association of orogenesis with subduction, as does the continuity of contemporaneous structural features across major dislocations. The style of basement thrusting and gravity modelling¹⁶, suggest that deformation was intracratonic and compressive. It was probably confined to the crust.

Palaeozoic deformation of the central Australian shield affected an area that was remote from the nearest continental margin. Lower Palaeozoic palaeomagnetic results from northern¹⁷, central¹⁸, and southern and western Australia¹⁹ suggest that these regions have not experienced large lateral displacements with respect to one another since Cambrian time. Furthermore, the northern and central Australian results indicate that these regions have acted as a single unit at least since the late Proterozoic. The orogenic events should be interpreted in terms of a single central Australian craton that underwent meridional compression while participating in the drift of Gondwanaland²⁰. The intermittent, north-south drift of Gondwanaland during the Palaeozoic is now well established^{21,22}. We suggest that rapid, intermittent drift of the large Gondwanide plate had intraplate orogenic repercussions, which are manifested in the prominent east-trending basement and cover structures in central Australia. Ancient zones of weakness in the crust imparted a mechanical anisotropy which provided loci for later thrusting; these may have coincided with old east-trending mobile belts now represented by deformed granulite zones. Recurrent thickening and warping of the crust¹³ produced the observed unconformities in the Proterozoic and Palaeozoic basins. When Australia drifted from Antarctica, it probably did so along one of the approximately east-trending ancient zones of weakness—perhaps an old mobile

belt, as has been suggested for the rifting of South America from Africa²².

There is clearly a need for a broader plate tectonic theory of orogenesis. Results from the Precambrian cratons of Africa and North America^{23,24} are consistent with the concept of intraplate orogeny. It seems that, within the limits of palaeomagnetic resolution, little relative motion has occurred between cratons and the intervening mobile belts have been interpreted as manifestations of ensialic tectonic phenomena. Accordingly, Piper *et al.*²⁴ suggested that a 'supplement' to the plate theory of Precambrian orogenesis is required. Glikson and Lambert²⁵ invoked a rift valley (aulacogene) mechanism to explain the development of certain West Australian mobile belts. We have further extended the plate theory of orogenesis to provide a viable basis for understanding the Palaeozoic deformed zones of central Australia.

Finally, it has been shown^{26,27} that continental plates, in particular those carrying North America and Australia, are in a state of compression under horizontal maximum principal stresses. Within such continents, earthquake shocks are generally caused by thrust faulting^{27,28}. Without invoking a driving mechanism, it is reasonable to expect that large deviatoric stresses existed in Palaeozoic continents and thus accounted for the deformed zones of central Australia.

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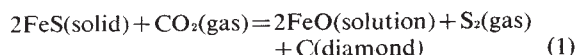
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Diamond growth by sulphide reduction of CO₂

MARX¹ proposed that natural diamonds were formed by a reduction of CO₂ and other theories have been proposed to explain their formation^{2–6}. Carbon dioxide is a common constituent^{7,8} and has been found as a gas in natural diamonds⁹. Marx proposed that pyrrhotite is active in the reduction process because it has been found as an inclusion in diamonds¹⁰. Furthermore, thermodynamic calculations showed such a reduction reaction to be energetically possible:



Diamond formation by this reaction probably would result in inclusions containing either free sulphur or sulphur compounds, or both. To test this theory experimentally, natural diamond was oxidised by pure oxygen at a high temperature. Any included sulphur would be converted to gaseous SO and SO₂ which could be detected by analysing the products with a research mass spectrometer. The instrument is about 10⁵ times more sensitive than any other spectroscopic method¹¹ and lends itself well to a study of minute quantities of a gaseous substance.

The mass spectrometer used was a 15.25-cm radius 90° sector type magnetic scanning instrument. The instrument, calibration and experimental procedures have been described¹².

Diamond fragments (16 g) were used⁹. The diamond was placed in a quartz tube which was evacuated by an oil diffusion pump to 10^{−7} torr. An oxygen atmosphere of 1 torr was then admitted to the sample tube. The sample was heated to a temperature of 1,050° C and maintained at that temperature for 45 min. The sample container was then placed in a dry ice–acetone bath and noncondensed components were removed by evacuation. Condensable substances were retained in the container. The remaining sample was analysed at room temperature using mass spectrometric techniques. Each experiment was duplicated three times. The weight of diamonds oxidised in each experiment was about 5.3 g.

No evidence for SO and SO₂ in the oxidation products was found in any of the three samples. The 16 g of oxidised diamond fragments comprised several hundred individual diamond crystals, some with visible inclusions, from Africa, Brazil and Arkansas. The detection sensitivity for sulphur was approximately 1 part in 10⁹ with respect to other oxidation products. Thus, there was no evidence for the presence of pyrrhotite. This suggests that the reduction of CO₂ by pyrrhotite is not responsible for the formation of all natural diamonds. These limited data do not, however, rule out the possibility of some diamond formation by the reduction of CO₂ by pyrrhotite.

BIOLOGICAL SCIENCES

E. coli lactose operon ribosome binding site

IN *Escherichia coli*, protein synthesis is initiated with formylmethionine, coded by the triplet AUG. As the first step in translation, ribosomes bind to the AUG initiator codon in the presence of initiation factors, charged fMet-tRNA and GTP. The sequence or structure of a messenger RNA molecule must signal that a particular AUG triplet is an initiation codon, and the cell's ribosomes must recognise this region as containing a signal for initiation of translation. In a reaction suitable for *in vitro* protein synthesis, except that it contains only one species of charged tRNA, fMet-tRNA, ribosomes bind to and protect initiation regions from nuclease digestion. The ribosomes bind at the AUG initiator codon and cannot proceed any further due to the lack of charged tRNA species other than fMet-tRNA. In the hope of determining the characteristics peculiar to an initiation region in a mRNA molecule, a number of ribosome binding sites have been sequenced^{1–8}.

I have previously reported the sequence of the first 63 bases of the lactose operon mRNA transcribed from the (CAP-independent) UV5 promoter mutant⁹. Figure 1 shows this sequence. The first AUG triplet occurs at positions 39–41, and the succeeding bases, read in triplets, code for the first seven amino acids of β-galactosidase¹⁰. This was highly suggestive that translation might initiate at position 39 of the UV5 *lac* message, and that ribosomes should bind to and protect the surrounding region of RNA from nuclease digestion.

To verify that the region around the AUG codon at position 39 is a ribosome binding site, I have isolated the portion of the UV5 *lac* mRNA which ribosomes protect from digestion by RNase A (which cuts after C and U) and RNase T₁ (which cuts after G). Table 1 lists the

Table 1 RNase T₁ oligonucleotides recovered after protection of a protein synthesis initiation complex from RNase A or T₁ digestion

Oligonucleotide	Molar yield after digestion with RNase A	Molar yield after digestion with RNase T ₁
G(A)	2-3	2-3
AUAACAAUUUCACACAG(G)	1/4-1/2	1
AAACAG(C)	1	1
CUAUG(A)	1	1
ACCAUG(A)	1	1
AUUACG(G)	1	1
AUUCACUG(G)	1/4	1/3-1/2

RNA was synthesised *in vitro* from UV5 *lac* DNA fragment template as previously described⁹. Synthesis was for 13 min at 25° C. After phenol extraction and two ethanol precipitations to remove unincorporated triphosphates, the ³²P-labelled RNA was taken up in 20 µl of 0.5 mM EDTA and incubated at 37° C for 15 min. Initiation complexes were formed in a 40 µl reaction mix similar to that described by Steitz¹ containing: 0.1 M Tris, pH 7.4, 0.05 M NH₄Cl, 5 mM MgOAc₂, 0.2 mM GTP, 2.5 mM β-mercaptoethanol, 6.6 A₂₆₀ units ribosomes, about 100 µg crude initiation factors, and 1.0 A₂₆₀ unit mixed tRNA charged with ³⁵S-formylmethionine. Ribosomes and initiation factors were prepared from *E. coli* B/r as described by Anderson *et al.*¹⁵, except that the preparative buffer contained 1 mM dithiothreitol instead of 6 mM β-mercaptoethanol; ribosomes were made up to 0.05 M Tris, pH 7.4 before the 2 M NH₄Cl wash; and initiation factors were dialysed into preparative buffer containing 5% glycerol, and made up to approximately 10% glycerol before storage at -70° C in plastic tubes. *E. coli* K12 mixed stripped tRNA (General Biochemicals) was charged with ³⁵S-methionine and formylated. Charging was assayed by precipitation in cold and hot TCA. After 12 min incubation at 37° C the reaction was cooled to 25° C, 75 µg ml⁻¹ RNase A or T₁ was added and incubation was continued at 25° C for 15 min. The reactions were layered on to linear 3.8 ml 5-20% sucrose gradients in the buffer described by Kondo *et al.*¹⁶, except that the MgOAc₂ concentration was raised from 5 to 10 mM. After centrifugation for 1 h at 55 K in the IEC SB405 rotor, about 20 fractions were collected directly into 3.5 ml scintillation vial inserts and the peaks of radioactivity determined by Cherenkov counts. Typically about 1-3% of the counts sedimented with the ribosomes after RNase digestion. These fractions were pooled, precipitated with 2.5 volumes ethanol and 1/10 volumes 20% NaAc at -20° C overnight, extracted with phenol, and precipitated with ethanol twice. The protected fragment was digested with RNase T₁ and fingerprinted by standard procedures described by Barrell *et al.*¹⁷. The data above are compiled from three separate experiments with A, C and U-labelled RNA.

oligonucleotides found in RNase T₁ fingerprints of this protected region. These oligonucleotides fit into the portion of the UV5 *lac* message surrounding the presumed initiator codon.

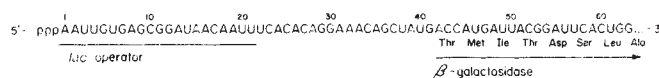
Table 2 compares the sequence of protected regions isolated after digestion with RNase A or RNase T₁. Ribosome protection does not preserve a unique sequence from nuclease attack: the size of the protected fragment depends on which nuclease accomplished the digestion, and on its concentration and activity. At the 3' end of the *lac*

ferred cleavage site in this region.

Sequences of nine other ribosome binding sites have been published¹⁻⁷, as well as that of an anomalous site on Qβ RNA which *B. stearothermophilus* ribosomes bind and protect⁸. A long stretch at the 5' end of MS2 RNA has also been sequenced, and this includes a region which probably contains the MS2 A protein initiation site; its sequence is very similar to that of the initiation region for R17 A protein, although the initiation codon is GUG^{11,12}.

Common features of initiation or ribosome binding regions are an AUG initiator codon, the sequence AGGA, the sequence PuPuUUUPuPu (Pu stands for purine), and one or more nonsense codons near the initiation site. Table 3 lists the sequences of the 11 known initiation regions, drawn so that similar sequences can be easily compared. All the initiation regions (or presumed initiation regions) which have been sequenced, except that for the MS2 A protein, include an AUG initiator codon. The *lac* ribosome binding site shares the sequence AGGA (positions 28 to 31 of the mRNA) with seven other ribosome binding sites^{1-8,11} and the *lac* site has the sequence AAUUUCA (positions 18 to 24) which, with C substituted for a purine, resembles the sequence PuPuUUUPuPu found in six other initiation regions^{1,4-6,11}. All but the *lac* site have a nonsense codon (UAA, UGA or UAG) within 15 bases of the initiator AUG; in *lac* the nearest nonsense codon (UAA) is 22 bases away. In some regions a stable conformation can be drawn with the AUG protruding at the 'ear' of a loop of RNA, but no such stable secondary structure is apparent in that of the *lac* operon. Table 3 also shows the site which *B. stearothermophilus* ribosomes protect on Qβ RNA⁸. This site probably contains the sequence AGGA, but it shares no other features with known initiation sites.

Though all these regions have a certain resemblance to one another, none of these common features alone, not even the AUG initiator codon, is an absolute requirement for ribosome binding. There is a GUG codon in the initiator region of the MS2 A protein¹². Reinitiations occur *in vivo* in mutants of the *lac i* gene (which codes for *lac* repressor) which carry early amber mutations. Three different mutant

**Fig. 1** The first 63 bases of the mRNA transcribed from the UV5 *lac* promoter⁹. The *lac* operator sequence is copied into bases 1 to 21. β-galactosidase initiates at the AUG codon at position 39, and the amino-terminal sequence of β-galactosidase¹⁰ is matched to the triplets on the message which code for those amino acids.**Table 2** Ribosome binding region of the *lac* message

Maximum RNase T ₁ -protected fragment:
5'-AUAACAAUUUCACACAGGAAACAGCUAUGACCAUGAUUACGGAUUCACUG
Minimum RNase T ₁ -protected fragment:
5'-AUAACAAUUUCACACAGGAAACAGCUAUGACCAUGAUUACG
Minimum RNase A-protected fragment:
5'-AGGAAACAGCUAUGACCAUGAUUACGGAU

Oligonucleotides recovered from ribosome-protected fragments of the UV5 *lac* message were fitted into the sequence of the first 63 bases of this messenger (shown in Fig. 1). This table compares the minimum site protected by RNases A and T₁ with the maximum protected region. Gel electrophoresis of protected fragments cleaved by RNase T₁ reveals two main species. Sequence analysis of these species show that they differ by a single oligonucleotide, AUUCACUG, which is reflected in the low recovery of this oligonucleotide after protection and digestion with RNase T₁ (Table 1). But gel electrophoresis of fragments protected from RNase A digestion reveals a range of species between 30 and 40 bases long, and no preferred cutting sites are evident in fingerprints of protected RNA.

ribosome binding site, the T₁ fragment AUUCACUG is sometimes protected and sometimes clipped during digestion with either RNase A or RNase T₁. At the 5' end the long T₁ fragment, AUAACAAUUUCACACAG, is always protected from RNase T₁ digestion, so its 3'-terminal G must be well shielded in the protected region. RNase A usually cleaves within this fragment, so some C and U residues at its 5' end must be exposed. It seems to have no pre-

ferred cleavage site in this region. proteins all have fMet as their amino-terminal amino acid, but these restarts occur at amino acid residues which are normally translated as formylmethionine (AUG), valine (GUX) or leucine (CUX, UUA or UUG), so ribosomes seem to bind to codons which resemble the normal AUG initiation site, and then mistranslate^{13,14}. Steitz has reported that *B. stearothermophilus* ribosomes bind *in vitro* to the RNA genome of the coliphage Qβ and protect a region

Table 3 Sequences of ribosome binding sites

AAACAUG <u>AGGA</u> UUACCAUGUCGAAGACAACAAAG	R17 replicase ¹
(AAC.U)A <u>AGGA</u> UGAAAUGCAUGUCUAAGACAGC	Q β replicase ³
AGAGCC(C)UCAACCGGGUUGAAGCAUGGCUUCUAAUUUACUCAG	R17 coat protein ¹
...AGAGCCUCAACCG(G.A)GUUUGAAGCAUGGCUUCCAACUUUACUCAG	f2 coat protein ⁵
AAUUUGAUC <u>AGG</u> CAAAAUUAGAGAC	Q β coat protein ²
CCU <u>AGGA</u> GGUUUGACCUAUGCGAGCUUUUAGUG	R17 A protein ¹
...CCU <u>AGGA</u> GGUUUGACCUUG	MS2 5' end ^{11,12}
GAGUAUAAG <u>AGGA</u> CAUAUGCCUAAAUAACCGCGUG	Q β A protein ⁴
AGGTTTTCTG.CTT <u>AGGA</u> .TTTAATCATGTTTCAGACTTTTATTTCTCGCCAC	Φ X174 spike protein ⁶
AACAUGAGGUAAACACCAAUGAUUUUACUAAAGAGCCUGCGAACG	a T7 <i>in vitro</i> RNA ⁷
AUAACA <u>UUU</u> UACACAC <u>AGGA</u> AACAGCUAUGACCAUGAUUACGGAUUCACUG	<i>lac</i> operon
AAACUG(AAAG.AG.G.G.G)AUUACACUCGAACGCCGCUAUCG	Q β - <i>B. stearothermophilus</i> ribosomes' site ⁸

Sequences of ribosome binding sites, from indicated sources, aligned with respect to their AUG or GUG initiator codons. Nonsense codons UAA, UGA and UAG are in italics. The popular sequence PuPuUUUPuPu is underlined, and AGGA is boxed. Parentheses and commas mark uncertainties in fragment order.

which has no initiator codon, although it probably does include the sequence AGGA⁸.

The *lac* operon ribosome binding site is the first to be sequenced from an *E. coli* operon. It is pleasing to find a close resemblance between phage and host protein synthesis initiation regions. But while there are similarities of sequence between many of the known ribosome-binding sites, the resemblance among all these sites is too casual to allow any conclusions about how ribosomes recognise initiation regions.

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Incorporation of exogenous DNA into mammalian chromosomes

MANY reports¹⁻⁴ have shown that exogenous DNA can be taken up and incorporated into the nuclei of cells in culture as well as *in vivo*; but no morphological evidence has been presented to suggest that such foreign DNA actually becomes associated with the chromosomes of inoculated cells. The Indian barking deer, *Muntiacus muntjak*, has the lowest number of chromosomes yet reported for mammals, $\varnothing = 6$; $\sigma = 7$ (ref. 5). The combination of such a low chromosome number and the unique morphology of each pair of chromosomes in the diploid set make this animal particularly attractive for comparative cytogenetic studies. The C- and G-banding patterns⁶⁻⁸ have also been established for this animal which allow even more detailed comparisons to be made than those based solely on gross chromosome morphology.

The purpose of this study was to determine if sequences of highly repetitive exogenous DNA could be incorporated into the muntjak chromosomes and demonstrated cytologically. Cells were inoculated with the synthetic polynucleotide poly (dA)·poly(dT) and then chromosome preparations were carried through the C-banding procedure which demonstrates repetitive DNA sequences⁹. The results suggest that, on the basis of new C bands, poly (dA) · poly (dT) can be incorporated into the muntjak genome (Table 1). The normal C-banding pattern is contrasted in Fig. 1 with a representative example of a cell showing extra C bands which presumably are due to new areas of highly repetitive DNA in the chromosome. Control cells (inoculation conditions 1 and 2) consistently showed a pattern of staining confined to the centromere regions of the autosomes, the centromere and neck of the X chromosome, and the entire Y chromosome. The new C bands were usually observed at the terminal portion of chromosome pair 1.

Seventy-two per cent of metaphases from cells inoculated with only Earle's balanced salt solution (EBSS) had the diploid set of chromosomes. The deviation from diploidy was due primarily to the loss of the small Y chromosome in many metaphases. This was also true for cells inoculated with DEAE · dextran poly(dA) · poly(dT), or the combination of the two. Extra C bands were observed only in the cells treated with poly (dA) · poly (dT) alone or in combination with DEAE · dextran and they occurred with approximately equal frequency in the two conditions.

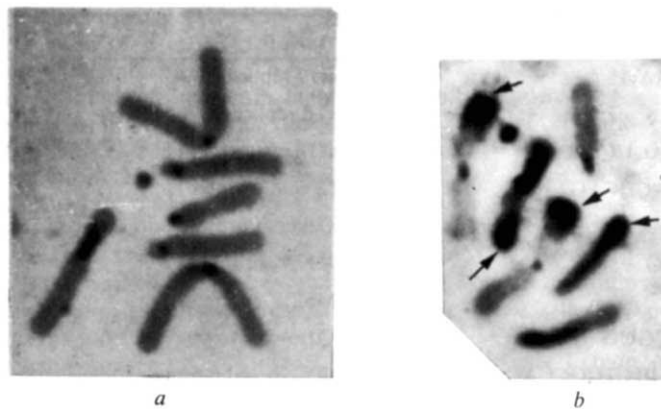


Fig. 1 C-banding patterns of *Muntiacus muntjak*. Cells were treated as described in Table 1. Chromosome spread (a) represents control cells inoculated with EBSS only (condition 1), and (b) was taken from cells inoculated with poly (dA) · poly (dT) (condition 3). Arrows point to extra C-bands as compared with controls.

Various studies^{1,3,4} have shown that exogenous DNA is taken up by mammalian cells and becomes intranuclear to the extent of from 0.5 to 5% of the host cell DNA, but breakdown of the exogenous DNA followed by resynthesis in the nucleus would lead to an apparent nuclear incorporation of the foreign DNA. It has been estimated that the amount of donor DNA that actually becomes incorporated into the chromosomal material of the inoculated cells is less than 0.3% of the host cell DNA³.

Our study avoids many of the inherent technical problems associated with previous biochemical and biophysical approaches to studying the incorporation of exogenous DNA into mammalian cells. Breakdown and reutilisation of the added poly (dA) · poly (dT) would not be expected to alter the C-banding pattern of the chromosomes, and only the portion of poly (dA) · poly (dT) that becomes associated with the chromosome would be detected by the C-banding technique. Since synthetic homoribopolynucleotides can be taken up by intact mammalian cells¹¹ without the addition of DEAE-dextran, it is not surprising that there was no difference in the

frequency of extra C bands between cells treated with poly (dA) · poly (dT) alone or in combination with DEAE dextran. The appearance of the extra C bands only at the terminal portion of the chromatids of specific chromosomes is of particular interest in that it suggests new DNA is added at specific sites and that certain chromosomes are more likely to be involved than others.

The observations reported here are consistent with the hypothesis that under certain conditions exogenous DNA can be taken up by cells and incorporated into the cell genome. An alternative explanation for the extra C bands is that poly (dA) · poly (dT) has indirectly induced the changes in banding patterns. Direct evidence for the incorporation of poly (dA) · poly (dT) into the chromosomes will require *in situ* hybridisation studies. If the new C bands prove to be of poly (dA) · poly (dT) origin and if they are heritable, it should be possible to use synthetic nucleic acids in gene therapy rather than viruses which carry unnecessary and often unwanted genetic information in addition to that which may be therapeutically useful.

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Table 1 Effect of synthetic DNA on chromosomes of *Muntiacus muntjak*

Inoculation	No. of cells examined	No. diploid (%)	No. with extra C bands (%)
1. EBSS	132	95(72)	0
2. DEAE-dextran	104	60(58)	0
3. poly(dA)·poly(dT)	263	162(62)	30(11.4)
4. DEAE-dextran + poly(dA)·poly(dT)	190	150(79)	19(10.0)

Fibroblast cells of the male muntjak were grown in medium MAB 87/3 (ref. 10) with 5% foetal bovine serum screened for coliphages. Slide culture chambers (Bio-Tek) were inoculated with cells and allowed to incubate at 37° C for 2 d to reach log phase of growth and about 1/2 confluency. Inoculation conditions included: (1) EBSS only; (2) DEAE-dextran (DEAE-D) (Pharmacia) (100 µg ml⁻¹) in EBSS; (3) poly (dA) · poly (dT) (PL Biochemicals) (2 µg ml⁻¹) in EBSS; and (4) DEAE-D (100 µg ml⁻¹) and poly (dA) · poly (dT) (2 µg ml⁻¹) in EBSS. Each culture was incubated with one of the above solutions for 15 min at room temperature, then rinsed once with Tris-buffered saline, and fed with 2.5 ml of complete growth medium. After about 36 h at 37° C, colcemide was added to a final concentration of 0.025 µg ml⁻¹ and cultures incubated an additional 16 h. Medium was then replaced by a hypotonic solution consisting of medium without serum:H₂O (1:2) for 20 min. Subsequent fixation was in acetic:methanol (1:3), and C-band staining was according to the procedure of Arrighi and Hsu⁹. C-band differentiation was enhanced by a rapid rinse in 50% methanol or a 5 min rinse in 10% methanol followed by a distilled H₂O rinse and air-drying. Metaphase spreads from each culture were then examined at 1,000× for exact count and C-banding patterns. The poly (dA) · poly (dT) used in these studies had a buoyant density of 1.644 in neutral CsCl, S_{20,w} = 7.9, and molecular weight = 5 × 10⁶.

Site of attachment of J chain to human immunoglobulin M

IN spite of a remarkable similarity of the general polypeptide chain structure of immunoglobulins, their molecular weights vary from 150,000 to 950,000 because some occur in monomeric (IgG, IgD and IgE) and others in monomeric or polymeric forms (IgA and IgM) (ref. 1). The largest immunoglobulin, IgM, appears in a stellate configuration of five disulphide-linked subunits (IgM₅), each consisting of two pairs of covalently linked heavy (µ) and light (κ or λ) chains². Recent evidence shows that polymeric immunoglobulins, whether composed of dimers, tetramers or pentamers, contain one molecule of an additional polypeptide, termed J chain³⁻⁵. Produced within the same cells as immunoglobulins, J chain becomes attached to polymeric IgM and IgA during the final stages of intracellular assembly⁶⁻⁸. Available results indicate that this polypeptide is essential for polymerisation of monomeric immunoglobulins^{9,10}, has a molecular weight of about 15,000 (refs 11-13), and is covalently attached by disulphide bonds to the Fc region of IgM (refs 14-16). Although the covalent structure of µ chain has been determined¹⁷, the exact location of the disulphide bond(s) connecting J chain to µ chain has not been known. To investigate this question, we used available information concerning fragmentation of IgM and of µ and J chains by cleavage with cyanogen bromide (CNBr) (refs 17-20). Because

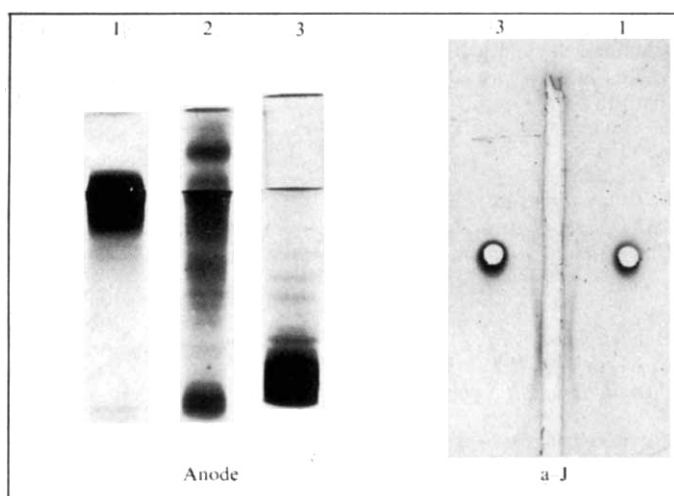


Fig. 1 Disc electrophoresis²² and immunoelectrophoresis with an anti-J chain serum (a-J) (ref. 23) of CNBr cleaved IgM (1), fraction A Fig. 2 (2), and fraction A purified by DEAE-ion exchange chromatography²³ (3). Lyophilised IgM was dissolved in 70% formic acid at 2% protein concentration and cleaved with CNBr for 4 h at room temperature. The amount of CNBr was double that of the protein. After dilution with ten volumes of distilled water, the material was lyophilised.

this procedure leaves disulphide bridges intact we reasoned that the portion of μ chain(s) linked by these bridges to J chain would be obtainable as a CNBr fragment.

IgM isolated from serum of a patient with Waldenström's macroglobulinaemia²¹ was cleaved with CNBr (ref. 20), and the products were examined by disc electrophoresis at pH 9.4 (ref. 22) and by immunoelectrophoresis with an antiserum to S-sulphonated J chain²³. CNBr treatment released a fragment with the electrophoretic mobility and antigenic determinants of J chain (Fig. 1). Purification of this fragment was achieved by extracting CNBr-cleaved IgM with a 1% ammonium bicarbonate buffer brought to neutrality by bubbling with CO₂. Whereas most large fragments are insoluble in this buffer and are removable by centrifugation, fragments containing J chain are soluble and can be further purified by gel filtration through Sephadex G-200 (Fig. 2) and subsequent DEAE-ion exchange

chromatography²³. Disc electrophoresis at pH 9.4 and polyacrylamide electrophoresis in the presence of sodium dodecylsulphate²⁴ showed the purity of this fragment, which reacted with an anti-J chain serum but not with antisera to IgM, or κ and λ chains (Meloy Laboratories).

The molecular weight of a purified J chain-containing fragment was established by sedimentation equilibrium ultracentrifugation in 5 M guanidine-HCl¹². The average molecular weight of three samples ($A_{280nm} = 0.4-0.7$) was 15,700 (range 15,400-15,900). Peptide maps and amino acid analyses²⁰ strongly resembled those of isolated J chain. This fragment, extensively reduced and alkylated, was applied on a column of Sephadex G-25, to separate individual components that were connected by disulphide bonds (Fig. 3). Resulting fractions were desalted, lyophilised, and their amino acids were analysed (Table 1). The first and largest fraction (B1) contained amino acids in proportions resembling those of J chain and its large N-terminal fragment²⁰. The second fraction (B2) contained amino acids found in a fragment obtained by CNBr cleavage of isolated J chain^{19,20}. The presence of a homoserine residue in B1 and its absence from B2 indicated that the former corresponded to the N-terminal and the latter to the C-terminal parts of J chain. The third fraction (B3) lacked homoserine, was eluted in the same position on Sephadex G-25 as the C-terminal octapeptide of α chain²⁰, and had an electrophoretic mobility and amino acid composition like that of the C-terminal octapeptide of μ chain^{16,25}. These results indicate that the peptide in fraction B3 originated from the C-terminal end of the μ chain and that J and μ chains are connected by a disulphide bridge between the penultimate cysteine residue of μ chain and a cysteine residue in the J chain. The molecular weight of the J chain-containing fragment implies the presence of one, perhaps two, octapeptides.

Cysteine residues involved in inter-IgM_s disulphide bonds are located at position 414 from the N-terminal end of the μ chain^{17,26,27}. Attachment of J chain to the penultimate cysteine

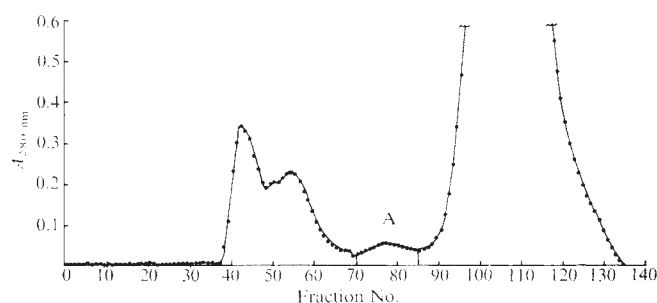


Fig. 2 Gel filtration of material obtained by extraction of CNBr-cleaved IgM with 1% ammonium bicarbonate solution saturated with CO₂ to neutrality. Extracted material was lyophilised, dissolved in 5 M guanidine-HCl, and applied on a column (2.6 × 100 cm) of Sephadex G-200 in 5 M guanidine-HCl. Material with electrophoretic mobility and antigenic determinants of J chain was present in fraction A (Fig. 1). Further purification was performed on DEAE Sephadex column²³.

residue (position 575) of only one or two C-terminal octapeptides indicates that J chain does not join individual IgM_s to form a pentameric molecule of IgM. If J chain, as well as previously described inter-IgM_s disulphide bridges, were to join five IgM_s to form a pentameric molecule, at least five of the ten C-terminal octapeptides would appear in association with J chain. In such a case a considerably increased molecular weight would be evident. Moreover, since at least two cysteine residues of J chain are involved in an intra-chain disulphide bridge²⁰, only four cysteine residues would be available to interact with IgM_s.

Of five known classes of human immunoglobulins, partial or complete amino acid sequences of the C-terminal parts of heavy chains have been determined for two monomeric immunoglobulins, IgG (ref. 28) and IgE (ref. 29), and for the two polymeric

Table 1 Amino acid compositions of J chain, purified fragment A and its components

Amino acids	J Chain IgM* in residues per 100	Fragment A in residues per 100	B2	JCNBr†	B3
			residues per chain		
Asp	16.5	16.1	2.0	2.0	1.0
Thr‡	9.0	9.1	2.1	1.9	2.0
Ser‡	5.8	6.4			0.9
Hse		0.7			
Glu	11.6	12.6	1.1	1.0	
Pro	6.6	5.8	2.3	1.9	
Gly	2.2	3.4			1.2
Ala	4.7	4.6	2.2	2.0	1.0
Val	7.1	6.5	0.8	0.8	
Met	0.7				
Ile	5.9	5.1			
Leu	6.5	6.6	1.2	1.0	
Tyr	4.2	3.1	0.7	0.8	0.6
Phe	1.2	1.3			
Lys	4.5	5.3			
His	0.9	1.1			
Arg	7.5	7.2			
Cys§	5.0	5.1	0.6	0.7	0.6

Amino acid analyses were performed on a Beckman 120 C amino acid analyser modified for single column, high speed analyses. Samples were hydrolysed in constant boiling HCl at 108°C for 24 h. All values represent the average of two or more analyses.

* Compiled from previously reported values^{4,13}.

† Obtained by CNBr cleavage of isolated J chain; small fragment separated on Sephadex G-25 (ref. 20).

‡ Corrected for losses due to acid hydrolysis.

§ Determined as cysteic acid²⁵.

immunoglobulins, IgM (ref. 17) and IgA (refs 30, 31). A comparison of the C-terminal portions of the heavy chains of these four classes revealed that α and μ chains extend beyond the C-terminal ends of the γ and ϵ chains by an additional 19 amino acids. The C-terminal octapeptides released by CNBr cleavage of μ and α chains are at the end of this extension^{17,30,31}. In polymeric IgA, J chain is also disulphide-linked to the penultimate cysteine residue of the α chain^{20,32}. The correspondence in sites of J chain attachment to μ and α chains and the pronounced homology of the two latter chains indicate that this region of both heavy chains is associated with the tendency of IgA and IgM molecules to polymerise.

phide bonding. With the polymerisation completed, J chain remains attached to the penultimate cysteine.

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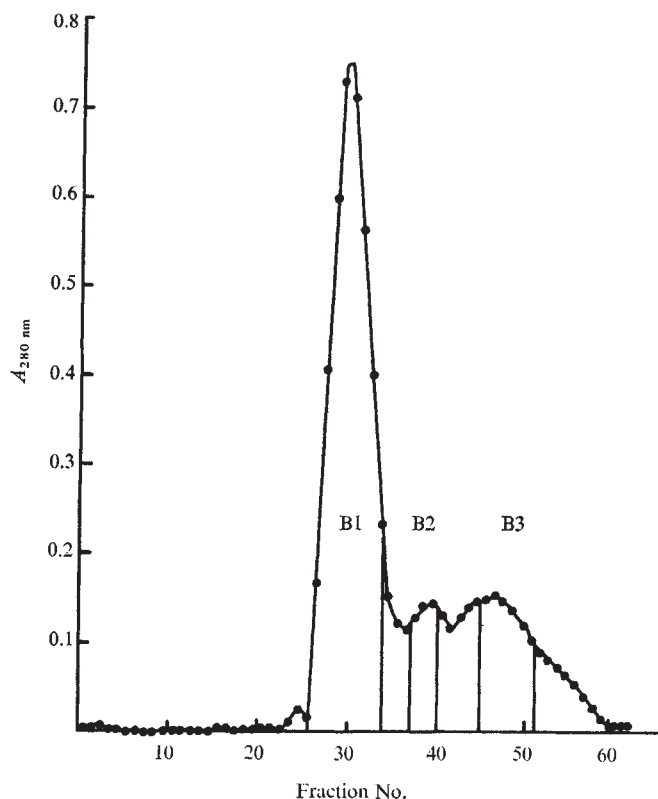


Fig. 3 Gel filtration of purified fraction A, reduced with 0.2 M 2-mercaptoethanol and alkylated with iodoacetamide²⁰. A column (1.5 × 35 cm) of Sephadex G-25 fine was equilibrated in 5 M guanidine-HCl. Fraction B2 was further purified by rechromatography on the same column. Desalting was performed on a Sephadex G-10 column in 1% ammonium bicarbonate buffer.

Nevertheless, the mechanism of polymerisation remains speculative. A successive disulphide bond interchange reaction initiated by J chain has been suggested^{5,13,33,34}. The incongruity between the necessity of J chain for polymerisation^{9,10} and the variant positions of the cysteine residues involved in the inter-IgM₂ disulphide bonds (residue 414) and that of J chain attachment (residue 575) may be resolved by postulating a labile disulphide bond between these residues in a monomeric molecule as suggested for IgA (ref. 30). CNBr cleavage failed to release two disulphide-linked C-terminal octapeptides that were released only after subsequent disulphide bond cleavage. This finding led to the conclusion that, rather than being mutually connected by a disulphide bond, the penultimate cysteine residues of the two α chains of monomeric IgA apparently form an intra- α or inter- α chain disulphide bond with the cysteine residue located elsewhere on the α chain. Possibly the penultimate cysteine residues of intracellular monomeric IgM are a part of an analogous labile disulphide bond that, if cleaved in the presence of J chain, might initiate the release of -SH groups that participate in inter-IgM₂ disul-

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Proportional increase of bursa-derived cells in chickens of the Obese strain

CHICKENS of the Obese strain (OS) spontaneously develop severe autoimmune thyroiditis during the first weeks of life, with clinical signs of hypothyroidism^{1,2}. Serological tests reveal both circulating and bound thyroglobulin autoantibodies³. The lymphoid infiltration of the thyroid gland is characterised by a predominance of plasma cells and the formation of a large number of germinal centres⁴. In view of the histological and serological features, the spontaneous autoimmune thyroiditis of OS chickens can be considered as an appropriate animal model for human Hashimoto thyroiditis⁵.

The major issue in research on autoimmune diseases is the question whether a primary autoantigenic stimulation (for example, by antigenically altered autologous material) of an essentially normal immune system leads to the development of disease, or if an abnormal immune system is incapable of maintaining the state of self tolerance. A combination of both mechanisms may also be involved in pathogenesis.

Investigations in the OS have provided data which support both a primary alteration of the thyroid glands before any signs of lymphoid infiltration do occur⁶, and a disturbed function of the immune system as well^{6,7}. With regard to the latter, it is noteworthy that the development of spontaneous autoimmune thyroiditis can be suppressed by neonatal or, even better, *in ovo* bursectomy⁸, whereas neonatal thymectomy leads to an increase of frequency and severity⁹ and also entails a significant augmentation of non-thyroid autoantibodies^{6,7,9}. In contrast to experimentally induced autoimmune diseases, the spontaneous autoimmune thyroiditis is therefore considered to be 'B dependent'. From these and other studies¹⁰, a new functional concept for the classification of autoimmune disease into B and T dependent extremes with intermediate forms has been proposed¹¹, and the diagnostic and therapeutic implications of this concept were discussed.

Table 1 Immunofluorescence tests on 10 NWL and 12 OS chickens at 19 d of embryonic life

Organ	Strain	% of lymphocytes reacting with:		
		ABS	ATS	alg
Bursa	OS	70	0	70
	NWL	71	0	57
Thymus	OS	2	92	0
	NWL	6	91	0
Spleen	OS	58	43	4
	NWL	29	64	1
Blood	OS	3	12	2
	NWL	ND	7	3

alg, Anti-whole-chicken immunoglobulin antiserum raised in rabbits. Figures indicate mean % of cells reactive with respective antisera. The lymphoid cells of each organ were pooled from all OS or NWL chickens. ND, not done

Here we describe studies on the distribution of B and T cells in various lymphoid organs of OS chickens by means of immunofluorescence tests with specific turkey anti-chicken bursa cell (ABS) and anti-thymus cell (ATS) sera¹². Antisera to chicken immunoglobulin have also been employed for the demonstration of immunoglobulin surface determinants as a further B-cell marker¹³. We thought that a postulated hyper-reactivity of the B-dependent portion of the immune system in the OS may be reflected in an altered distribution of B and T cells in the chickens' lymphoid tissues.

Table 2 Immunofluorescence tests on 5 NWL and 4 OS chickens 1.5 weeks after hatching

Organ	Strain	% of lymphocytes reacting with:			
		ABS	ATS	algM	algG
Bursa	OS	85 ± 3	8 ± 2	67 ± 8	22 ± 9
	NWL	74 ± 7	10 ± 6	62 ± 11	17 ± 14
Thymus	OS	28 ± 5	63 ± 11	ND	ND
	NWL	11 ± 6	87 ± 8	ND	ND
Spleen	OS	58 ± 6	41 ± 5	50 ± 14	5 ± 1
	NWL	19 ± 4	56 ± 9	12 ± 4	6 ± 6
Blood	OS	27 ± 2	63 ± 5	19 ± 6	4 ± 3
	NWL	21 ± 5	68 ± 3	18 ± 5	1 ± 2

algM, Anti-chicken-IgM-serum raised in rabbits; algG, anti-chicken-IgG-serum raised in rabbits. Cell suspensions were not pooled. Figures indicate mean and s.d. ND, not done.

Lymphoid cells from the bursa of Fabricius, thymus and spleen were prepared by consecutively passing tissue fragments through 80 and 200 mesh screens and washing the cell suspensions in phosphate buffered saline (pH 7.2). Blood lymphocytes were obtained by sedimentation in polyvinylpyrrolidone (PVP) (refs 12, 13). The viability of the cells was always >90%. The reaction of cells with ABS or ATS was visualised in an immunofluorescent staining procedure on living cells¹⁴. The FITC-labelled antiserum to turkey immunoglobulin used in the indirect immunofluorescence tests was produced in our laboratories and characterised as described earlier¹². Appropriate control experiments to exclude nonspecific staining were also carried out^{12,13}. The conjugate was absorbed with chicken immunoglobulins. All readings were done on coded slides and with epi-illumination on a Reichert Zetopan microscope equipped with a HBO-200 mercury vapour lamp and suitable exciter and barrier filters¹². After the immunofluorescent staining procedure the cells were counterstained with Evans blue to facilitate counting. IgM and IgG determinants on the surface of lymphocytes were detected in an indirect immunofluorescent test using specific rabbit antisera prepared as described elsewhere (B.A. and G.W., unpublished).

Table 1 gives the results obtained with cell suspensions of 19-d-old embryos. At this age there is a greater percentage of B cells in the spleen of OS chickens than in the spleen of NWL controls. No such feature was found in the other lymphoid populations tested. Using an anti-whole-immunoglobulin serum, there seems to be an increase of cells with immunoglobulin surface determinants (ISD) in the OS chicken bursa as compared with NWL birds.

Table 2 summarises the data from chickens 1.5 weeks after hatching. At this age an increase of B cells is not only found in the spleen but also in the thymus of OS as compared to NWL birds. Analysis of the cell populations

Table 3 Immunofluorescence tests on three NWL and three OS chickens 4 weeks after hatching

Organ	Strain	% of lymphocytes reacting with:				
		ABS	ATS	alg	algM	algG
Bursa	OS	91 ± 6	1 ± 3	76 ± 2	65 ± 15	29 ± 3
	NWL	86 ± 7	4 ± 2	91 ± 9	44 ± 4	42 ± 11
Thymus	OS	25 ± 6	68 ± 5	20 ± 3	15 ± 3	7 ± 1
	NWL	3 ± 3	93 ± 4	4 ± 2	1 ± 1	1 ± 2
Spleen	OS	57 ± 4	33 ± 6	53 ± 7	33 ± 8	15 ± 2
	NWL	28 ± 7	52 ± 9	35 ± 5	15 ± 2	18 ± 8
Blood	OS	21 ± 3	68 ± 7	16 ± 7	11 ± 5	9 ± 2
	NWL	23 ± 5	66 ± 6	19 ± 3	14 ± 9	10 ± 6

See legend for Tables 1 and 2

with chain specific anti-immunoglobulin sera revealed a preponderance of IgM over IgG-carrying B cells in the spleen of OS chickens. The additive values obtained with anti-IgM and anti-IgG were in good agreement with the figures obtained by incubating the cells with ABS. Four weeks after hatching a similar pattern was obtained (Table 3), but now the ratio of IgM to IgG carrying cells was also higher in the bursa of the OS as compared to NWL controls.

Table 4 shows that in 9-week-old OS chickens the proportion of B cells is increased in spleen, thymus and blood, and that here again most of these cells bear IgM on their surface. From the data obtained with chain-specific anti-immunoglobulin sera it seems reasonable to assume that the increment in B cells in lymphoid organs of the OS chickens is made up of IgM-ISC carrying cells. As in the 4-week-old animals, this IgM shift is also reflected in the bursal cell population.

A crucial observation in these studies was the altered splenic proportion of B and T cells already in OS embryos, that is, well before any signs of thyroiditis can be found. Thus, this increase of B cells in OS birds as compared with NWL chickens seems to be independent of the thyroiditis-induced metabolic or endocrine status. The occurrence of nearly 30% of B cells in the thymus of 1.5-week-old OS birds again emphasises this point. In NWL chickens such high numbers of B-cells in the thymus are only rarely attained, and if they do occur at all in healthy, unimmunised birds, they are found much later in life¹⁵.

It may be hypothesised that the abnormal invasion of the OS thymus by B cells may impair normal maturation and regulatory processes in it, or that some primary derangement of the thymus function is a prerequisite for such an abnormal immigration of B cells. The late appearance of an increase in B cells in OS chicken blood may be

this strain may be a deficient T-cell depressor compartment². In addition, the present approach may not only provide information about diagnostic aspects of autoimmune disease but also entail the use of appropriate T or B-cell specific immunosuppressive procedures. In the case of the OS, this point has already been substantiated by suppressing the spontaneous autoimmune thyroiditis with anti-bursa cell¹⁶ or anti-immunoglobulin sera (G.W., B.A. and W. Johnson, unpublished). That this concept may also be true for human autoimmune diseases was confirmed in recent studies by Chused *et al.*¹⁹ who demonstrated a significant increase of B cells in Sjögrens syndrome.

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Table 4 Immunofluorescence tests of four NWL and four OS chickens at 9 weeks of age

Organ	Strain	% of lymphocytes reacting with:			
		ABS	ATS	algM	algG
Bursa	OS	92±6	7±3	71±12	35±6
	NWL	91±8	6±5	48±9	55±6
Thymus	OS	32±6	67±13	22±5	9±4
	NWL	12±8	86±5	4±2	7±3
Spleen	OS	60±9	40±6	39±3	26±8
	NWL	38±5	58±8	15±6	22±4
Blood	OS	56±7	26±8	34±7	14±5
	NWL	23±6	70±11	12±4	10±3

See legend for Tables 1 and 2

explained either by the fast migration of 'pathological' B cells from blood to the tissues and thus the maintenance of normal levels of B cells in the blood for a longer period until the capacity of the peripheral lymphoid tissues for B cells is saturated, or by a selective microenvironment specifically attracting B cells into the prospective lymphoid organs early in the animals development.

Attempts to delineate B and T cells in NZB mice, the other well established model of spontaneous autoimmune disease, gave ambiguous results^{10,17}. An alteration of the proportion of T cells to B cells has only been observed very late in the animals life, after the onset of clinical symptoms of the disease.

Our results are the first step towards a diagnostic application of the concept of B and T dependent autoimmune diseases^{10,11}. As stated above, this concept is only concerned with the effector cells, and, in the case of OS chickens, the postulated hyper-reactivity of the B cell system does not exclude the possibility that the initial aetiological factor in

Reversal of immunological tolerance by cyclophosphamide through inhibition of suppressor cell activity

It has been shown in mice that specific immunological unresponsiveness to contact sensitisation with picryl chloride induced by pretreatment with picryl sulphonic acid is a positive phenomenon rather than an absence of specific immunocompetent cells¹. In this system it was shown that transfer of lymph node cells from unresponsive to normal mice would limit the recipients' contact sensitivity response to picryl chloride and that these cells had characteristics suggesting that they were T as opposed to B cells². We should like to report that a similar state of unresponsiveness to the dinitrophenyl group in guinea pigs can be broken by treatment with cyclophosphamide (CY) just before sensitisation and this is associated with the return of the ability of T cells to proliferate in the draining lymph node as part of the immune response.

The guinea pigs used were of the outbred white spotted Himalayan strain (400–450 g). Tolerance was induced by two intravenous injections of 500 mg kg⁻¹ dinitrobenzene-

Table 1 Reversal of tolerance to dinitrophenyl hapten by cyclophosphamide

Experimental protocol			Mean skin reactivity				PCA titre*
		Time after sensitisation (d)	14	28	56	70	
1	SO ₃ -SO ₃	DNFB (CFA)	0 (6)	0 (6)		0 (6)	0 (6)
2	SO ₃ -SO ₃ CY (3 d)	DNFB (CFA)	1.25 (6)	1.0 (5)		1.0 (5)	1:22 (6)
3	SO ₃ -SO ₃ CY (14 d)	DNFB (CFA)	0 (6)		0 (6)		1:7 (6)
4		DNFB (CFA)	1.9 (6)				1:30 (7)
5	SO ₃ -SO ₃	DNCB (contact)	0 (6)	0 (6)		0 (6)	
6	SO ₃ -SO ₃ CY (3 d)	DNCB (contact)	1.1 (5)	0.5 (5)		0.75 (4)	
7		DNCB (contact)	1.3 (8)				

* 21 d after sensitisation.

SO₃-SO₃ indicates DNBSO₃ given at intervals of 14 d. In experiments 1, 2, 5 and 6 sensitisation was with DNFB in CFA or contact with DNCB 14 d after the last dose of DNBSO₃. In experiments 2 and 6 sensitisation was preceded by CY, 3 d previously. In experiment 3 sensitisation was with DNFB in CFA 28 d after the last dose of DNBSO₃ and was preceded by CY, 14 d previously. The figures in parentheses refer to the number of animals in each experiment.

sulphonic acid sodium salt (DNBSO₃) with an interval of 14 d, the last dose being 14 or 28 d before an attempt at sensitisation with 500 µg of 2, 4-dinitrofluorobenzene (DNFB) in complete Freund's adjuvant (Difco) (CFA) or contact with 50% 2, 4-dinitrochlorobenzene (DNCB) in acetone on the ear. Sensitivity was tested by contact with 0.09, 0.05 and 0.03% DNCB in acetone on the flank. The intensity of skin reactions was assessed 24 h later as described previously³. Animals were injected intraperitoneally with 250 mg kg⁻¹ CY either 3 or 14 d before sensitisation. Antidinitrophenyl antibodies were detected three weeks after sensitisation by passive cutaneous anaphylaxis¹. Auricular lymph nodes draining the site of application of a sensitising dose of DNCB 4 d previously were examined for immunoblast (large pyroninophilic cell) proliferation in paracortical areas by counting the number of these cells in a microscopic field of diameter 450 µm. At least 10 lymph nodes were examined in each group.

Table 1 shows that CY given 3 d before sensitisation, 14 d after the last dose of DNBSO₃ effectively reverses the unresponsiveness of guinea pigs to DNFB or DNCB. Reversal of unresponsiveness was found both with contact sensitivity (a cell-mediated immune phenomenon) and in the production of humoral antibodies detectable by PCA. Reversal of unresponsiveness in contact sensitivity persisted beyond 70 d after immunisation and therefore was not a temporary phenomenon. But if sensitisation was not attempted until 14 d after CY, no reversal of tolerance could be detected in contact sensitisation although low titres of PCA antibody were produced. This would indicate that sensitisation had to be within a limited period after CY to be fully effective.

A comparison of the effect of these various measures on T-cell proliferation in the lymph node draining the site of sensitisation is shown in Table 2. It can be seen that the development of unresponsiveness by treatment with DNBSO₃ is associated with an eight-fold reduction in the number of immunoblasts seen 4 d after sensitisation with DNCB. Treatment of these DNCB unresponsive animals with CY 3 d before sensitisation was associated with a four-fold rise in the number of immunoblasts to the level found in control animals given CY 3 d before sensitisation. But if sensitisation was delayed to 14 d after CY, the level of immunoblasts in the draining node did not return to even half the level found in control animals sensitised 14 d after CY, which had not been previously made unresponsive with DNBSO₃. These findings seem to parallel the peripheral manifestations of the immune response. The return of ability of unresponsive animals to react is associated with a reversal of the depression of the ability of T cells to proliferate in the draining lymph nodes following sensitisation. The failure to show reversal of unresponsiveness if sensitisation is delayed 14 d after CY corresponds with an inability to obtain a level of immunoblast proliferation nearer to that found in control sensitised animals.

The increased proliferation of T cells in tolerant animals treated with CY just before sensitisation cannot be simply a rebound phenomenon, since in normally sensitised animals pretreatment with CY results in a significant decrease in immunoblasts in the draining node.

The preferential inhibition of suppressor cell activity by CY has been demonstrated previously in normal immune responses. These include contact sensitivity and the Jones-Mote type of delayed hypersensitivity in the guinea pig⁵⁻⁷. Cyclophosphamide seems to act on immunological mechanisms by depressing B lymphocytes in preference to T lymphocytes and is selectively cytotoxic to those cells which are short lived and have a more rapid rate of turnover in peripheral lymphoid tissues⁸⁻¹⁰. These changes have been demonstrated both histologically and by the use of techniques to detect the θ antigen on lymphocytes on the mouse as well as by labelling studies using ¹²⁵IUDR and ⁵¹Cr. As it can be reversed by treatment with CY, specific immunological tolerance might be considered to be the result of active suppression of the ability of T cells to proliferate in lymphoid tissue. These observations also indicate that, in this model, active suppression of T-cell function is on the afferent rather than the efferent side of the immune arc. Moreover we have observed that tolerance to DNCB can be reversed in syngeneic animals by transfusion of cells from actively sensitised donors. This is a further indication that suppression does not occur on the efferent side of the arc¹¹. The finding that CY reverses tolerance if animals are sensitised 3 d later effectively excludes the role of enhancing antibody or circulating immune complexes in this system, as antibody levels would not be significantly reduced such a short time after treatment.

It seems therefore that CY has a preferential action on clones of suppressor cells, releasing effector cells for proliferation in cell-mediated immunity and in antibody production. Our results also show that suppressor cells can regenerate within 2 weeks of CY treatment, so that sensitisation by DNFB at this time fails. In spite of previous observations that CY acts preferentially on B rather than

Table 2 Cytology of lymph nodes draining site of application of 50% DNCB 4 d previously

Experimental protocol			Immunoblasts in paracortical area field* 450 µm diameter
1	SO ₃ -SO ₃	DNCB	24.5 ± 9.9
2	SO ₃ -SO ₃ CY (3 d)	DNCB	108 ± 48
3	SO ₃ -SO ₃ CY (14 d)	DNCB	64.5 ± 29
4		DNCB	134 ± 46
5	CY (3 d)	DNCB	169 ± 59.4
6	CY (14 d)	DNCB	194 ± 57

* Arithmetical mean ± s.d.

Significance of differences 1+2, $P < 0.001$; 2+4, P : no significant difference; 3+5 $P < 0.001$; 4+6, $P: 0.01 > P > 0.002$.

Notation of experimental protocol is as in Table 1.

T cells, our present observations do not exclude a role for short lived rapidly proliferating suppressor T cells that might be affected by CY in the same way as B cells and differently from effector T cells. At present one cannot say whether in this system the suppressor cells belong to a T or a B cell population.

A similar reversal of homograft tolerance has been described following irradiation in mice¹². But in this and other reports the effect can only be obtained in weak or already terminating states of tolerance¹³.

In conclusion we suggest that CY can break immunological tolerance by inhibition of a population of suppressor cells that effectively prevent T-cell proliferation in the draining lymphoid tissue and thus prevent the release of specifically sensitised cells into the periphery. Whether these suppressor cells act by preventing antigenic recognition or by direct inhibition of the proliferative phase of the immune response has not as yet been determined.

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by a peripheral histocyte-mediated destruction of tumour cells⁸. We have now examined the effect of intradermal BCG on the 'specific' and 'nonspecific' responsiveness of circulating lymphocytes. We also investigated the ability of two chemical agents to produce similar or additive effects.

Strain 2 guinea pigs were inoculated intradermally with either BCG or dinitrochlorobenzene (DNCB), or nitrogen mustard, or all three of these agents. Each agent produced a local delayed hypersensitivity reaction. BCG was given as an 0.1 ml inoculation of *Mycobacterium bovis* vaccine (Research Foundation, Chicago, Illinois). DNCB was given as an 0.1 ml intradermal inoculation containing 2,000 µg dissolved in acetone. Nitrogen mustard was administered as an 0.1 ml intradermal inoculation of 100 µg of Mustargen (Merck, Sharpe and Dohme) in physiological saline. Animals that

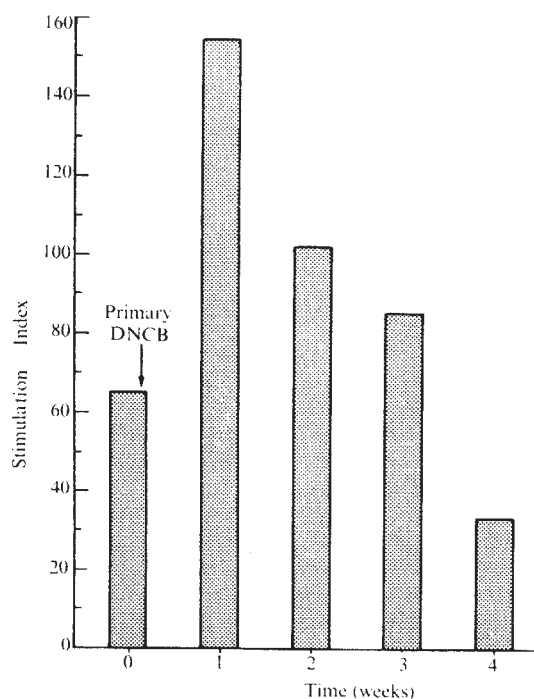


Fig. 1 Effect of primary DNCB exposure on lymphocyte responsiveness to PHA in 13 patients

received all three agents received three separate intradermal inoculations.

Six weeks later, blood was drawn by cardiac puncture and lymphocytes were separated on Ficoll-Hypaque gradients and tested for reactivity against phytohaemagglutinin (PHA) and tuberculin purified protein derivative (PPD) using a microtechnique⁹. Two hundred thousand lymphocytes per well were incubated in a Falcon Microtest plate for 48 h with 10 µg of PHA or PPD, followed by a 24 h incubation with 2 µCi of ³H-thymidine, and then liquid scintillation counting of the d.p.m. of the ³H-thymidine taken up by cellular DNA. The same assay was used to test guinea pig, monkey and human circulating lymphocytes. Slightly different conditions were used to test mice, since lymphocytes were of splenic origin, and were incubated with PHA for shorter periods¹⁰.

Table 1 shows a typical experiment in which guinea pigs treated with BCG had a higher responsiveness to PHA than did guinea pigs not treated with BCG. The same is true for reactivity against PPD. Pigs treated with a combination of BCG, DNCB and nitrogen mustard had the highest reactivities to PHA and PPD. Other studies showed that intradermal DNCB or nitrogen mustard alone also increased PHA responsiveness. Similarly, in mice, increased PHA reactivity of lymphocytes was observed in animals inoculated subcutaneously 10 d previously with 1,000–2,000 µg of DNCB.

Augmentation of lymphocyte reactivity in guinea pigs, mice, monkeys and humans sensitised to BCG, dinitrochlorobenzene or nitrogen mustard

NONSPECIFIC augmentation of tumour immunity has been achieved through the use of *Bacillus Calmette-Guérin* (BCG), *Corynebacterium parvum*, *Toxoplasma gondii*, *Besnoitia jellisoni* and other intracellular parasites¹⁻⁴. Nonliving preparations, such as cell walls from BCG, also cause nonspecific augmentation of tumour immunity⁵. The mechanisms underlying these effects are not clear. It has been postulated that amplification of macrophage or reticuloendothelial function is responsible for the enhancement of tumour immunity by these agents. In the mouse, BCG causes a change in the rate of activation of macrophages, as well as a change in their lysosomal content and bacteriolytic power^{6,7}. In the guinea pig, there is an initial granulomatous reaction at the BCG inoculation site, followed

Table 1 Effect of intradermal BCG, DNCB and nitrogen mustard on guinea pig lymphocyte stimulation by PHA and PPD

Sensitisation	Media	³ H-Thymidine (d.p.m.)			Stimulation index of circulating lymphocytes	
		PHA	PPD		PHA	PPD
Nothing	3,478 ± 428	4,869 ± 308	2,670 ± 74		1.4	0.8
BCG	3,769 ± 726	11,852 ± 1,845	6,834 ± 1,300		3.1	1.8
BCG, DNCB and nitrogen mustard	4,774 ± 1,282	17,023 ± 832	11,385 ± 3,797		3.6	2.4

The possibility of enhancement by DNCB and nitrogen mustard of BCG induced-lymphocyte responsiveness was then examined in seven rhesus monkeys. Two monkeys had received no previous BCG and were PPD negative. Five monkeys had been inoculated intradermally with 10⁷ viable BCG organisms every 2 to 3 weeks for 4 months. On repeated skin testing with second strength PPD, two of the latter five had developed a positive PPD reaction, and two were negative to intradermal PPD in spite of repeated BCG administration. Blood lymphocytes from all these animals were tested for PHA and PPD reactivity (Table 2).

responsiveness after the first exposure to DNCB. The average stimulation index 4 weeks after primary sensitisation was less than the average value before sensitisation. This may be due to an undershoot following the peak stimulation, or may reflect a lack of stability in this population of cancer patients, several of whom underwent surgery, radioisotopic scans or other diagnostic procedures during the interval of study. These factors are being evaluated. Patients exposed a second time to DNCB also showed enhancement of PHA responsiveness.

Our studies support the conclusion that agents that produce a delayed hypersensitivity reaction increase PHA responsiveness

Table 2 Effect of BCG treatment on monkey lymphocyte stimulation by PHA and PPD

Monkey no.	BCG treatment every 2-3 weeks × 4 months	Skin reactivity to PPD	Stimulation index of circulating lymphocytes	
			PHA	PPD
50	No	Neg.	6.0	0.86
605	No	Neg.	8.2	1.6
248	Yes	Neg.	21.3	6.0
922	Yes	Neg.	33.0	4.0
676	Yes	±	18.0	7.3
730	Yes	Pos.	8.5	4.3
708	Yes	Pos.	14.1	19.0

As expected, the PPD stimulation index was lowest in monkeys receiving no BCG (0.86–1.6), and considerably higher in treated monkeys (4.0–19.0). As was true in other species, the PHA reactivity was also enhanced by BCG, varying from 8.5 to 33.0, compared with 6.0 and 8.2 in the two control monkeys.

Four of the monkeys were then randomly chosen for intradermal inoculation with 0.2 ml of DNCB and 0.2 ml of nitrogen mustard. The preparations were the same as described above for the guinea pigs. When blood lymphocytes were tested 1 week later, there was an average increase of 106% in the PHA stimulation index and 221% in the PPD stimulation index. In two animals not treated with DNCB and nitrogen mustard, the average PHA and PPD stimulation indices fell 11% and 24% respectively. These data are summarised in Table 3.

of the host's circulating lymphocytes. This enhancement of peripheral lymphocyte reactivity may be a primary pathway by which BCG is effective in tumour immunotherapy. As in some forms of chemotherapy, the combination of agents seems more effective than either agent alone. The application of this observation to immunotherapy and tumour-specific immunity is being examined.

During immunological monitoring the effect of one test on the results of another should be considered, as well as the effect of the test on the patient's immune status. Reactivity to DNCB has been found to be a favourable prognostic sign in patients undergoing surgery for malignancy¹¹. It is also conceivable that the nonspecific stimulation of circulating lymphocytes produced by DNCB sensitisation has some adjunctive therapeutic value

Table 3 Effect of DNCB and nitrogen mustard on BCG-induced enhancement of lymphocyte responsiveness

Monkey no.	DNCB and nitrogen mustard	Stimulation index		% Change in stimulation index	
		PHA	PPD	PHA	PPD
730	No	3.4	2.5	– 60%	– 42%
605	No	11.4	1.5	+ 39%	– 6%
248	Yes	84.0	41.3	+ 300%	+ 583%
708	Yes	27.3	10.0	+ 93%	– 47%
922	Yes	26.7	9.8	– 19%	+ 145%
676	Yes	27.1	22.1	+ 51%	+ 203%

In the final monkey experiment, three monkeys (Nos 708, 922 and 676) which had previously received BCG, DNCB and nitrogen mustard were given all three agents again. Their average PHA stimulation index 1 week later was 63, representing average increases of 133% over the immediately previous values and 190% over the initial values. The largest increase of the three was in monkey 708, which was the only PPD positive animal in that subgroup.

In patients with or without previous BCG treatment, we have observed that topical application of 2,000 µg of DNCB increases PHA reactivity of circulating lymphocytes. The data from 13 patients with melanoma, sarcoma and adenocarcinoma are presented in Fig. 1. Although no patient was tested at every point, the composite of all the values shows a doubling of PHA

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Transplantation of allogeneic lymphoid cells specifically depleted of graft versus host reactive cells

GRAFT versus host (GvH) disease has been a major problem in the transplantation of allogeneic haematopoietic or immunocompetent lymphoid cells. In man, attempts to reduce the GvH effect of transplanted allogeneic cells, including HL-A matching^{1,2}, cell separation techniques³, anti-lymphocyte globulin⁴, and injection of small doses of bone marrow cells⁵, have aided successful bone marrow transplantation. Various techniques have been used in animal systems⁶⁻¹⁴ to reduce the GvH reaction by removing reactive cells from populations of lymphoid cells. So far, however, there has been no attempt to assess the potential value of such specifically depleted cells in transplantation.

Recently we have shown that one of these techniques, adsorption of immune cytotoxic thymus-derived (T) cells onto monolayers of target cells bearing the sensitising antigens, is greatly improved by centrifugation¹⁵, thus giving 70-90% depletion of cytotoxic cells. These results encouraged us to apply this fractionation technique to deplete nonimmune lymphoid precursor cells carrying surface receptors for mouse alloantigens. We have now found that normal BALB/c (H-2^b) spleen cells can be depleted specifically of GvH reactive cells (against histoincompatible C57BL/6 (H-2^b) alloantigens), and that lethally irradiated C57BL/6 mice transplanted with the fractionated BALB/c allogeneic cells survive with no apparent signs of GvH disease.

Fractionation of normal BALB/c spleen cells on C57BL/6 spleen cell monolayers abolished their capacity to become sensitised *in vivo* against C57BL/6 alloantigens as measured *in vitro* by the ⁵¹Cr release assay using EL-4 (H-2^b) labelled target cells (Table 1). The specificity of the adsorption was investigated using monolayers prepared with antigenically unrelated cells. BALB/c spleen cells which had been adsorbed on either BALB/c or SJL/J (H-2^k) monolayers or on empty dishes (not shown) retain the ability to become sensitised against C57BL/6 and elicit cell-mediated cytotoxicity (CMC) *in vitro* (Table 1, experiment 2). These results are consistent with the concept that precursor cells for CMC, known to be T cells^{15,17,18}, possess receptors on their surface which can recognise and bind specifically to the corresponding cell surface antigens.

We next investigated whether the fractionated spleen cells can reconstitute lethally irradiated allogeneic recipients in the absence of developing fatal GvH disease. C57BL/6 mice injected with: (1) unfractionated BALB/c spleen cells; (2) BALB/c spleen cells adsorbed either on BALB/c or on SJL/J spleen cell BALB/c monolayers, and (3) spleen cells adsorbed on empty plastic dishes, all developed GvH disease characterised by runting, and died within 2-4 weeks of inoculation. On the other hand, about 70% of the C57BL/6 mice that received 4-6 × 10⁷ BALB/c spleen cells adsorbed on C57BL/6 monolayers survived more than 6 months with no signs of disease (Table 2). Control lethally

Table 1 Failure of *in vivo* sensitisation of BALB/c spleen cells adsorbed onto C57BL/6 spleen cell monolayer

Experiment	No. of BALB/c donor cells	Spleen cell monolayer used for adsorption	Cell-mediated cytotoxicity at effector target ratio of	
			100:1	25:1
1	6 × 10 ⁷	None C57BL/6	45.8 ± 0.9 -0.2 ± 0.4	39.5 ± 1.6 -0.06 ± 0.01
2	5 × 10 ⁷	None C57BL/6 (H-2 ^b) BALB/c (H-2 ^d) SJL/J (H-2 ^k) None	30.0 ± 0.04 1.4 ± 0.04 29.2 ± 0.8 25.2 ± 0.4 -0.8 ± 0.27	23.2 ± 1.8 0.02 ± 0.6 15.2 ± 1.4 21.9 ± 2.4 -1.63 ± 0.4

Donor cells from 10-12-week-old male BALB/c mice were teased and a cell suspension was prepared in Eagle's minimal essential medium (MEM). Various numbers of fractionated or unfractionated lymphoid cells were injected intravenously into lethally irradiated (750-800 r., using a ⁶⁰Co source, 35 r. min⁻¹) male C57BL/6 mice of the same age. The mice were irradiated 2 h before inoculation and injected intraperitoneally with heparin (50 U) 15 min before cell administration. Antibiotics were included in the drinking water for the first 3 weeks after inoculation. Six (experiment 1) or 7 d (experiment 2) after inoculation mice were killed and their spleens were tested *in vitro* for cell-mediated cytotoxicity against ⁵¹Cr-labelled EL-4 (H-2^b) target cells as described before¹⁸. Spleen cell monolayers used for adsorption were prepared by the technique described by Stulting and Berke¹⁶, using poly-L-lysine (Sigma)-coated polystyrene Petri dishes (60 × 15 mm, Falcon No. 3002). Briefly, 8 × 10⁷ to 10 × 10⁷ (2 ml) spleen cell leukocytes suspended either in Dulbecco's phosphate-buffered saline (PBS) or in RPMI 1640 medium were added to each plate. The plates were incubated at room temperature for 45 min and then washed thoroughly with PBS. Adsorption was performed as described before¹⁵. 2 × 10⁷ (2 ml) BALB/c spleen cells were added to each monolayer and incubated at 37° C for 45 min in a humidified atmosphere of 5% CO₂ and 95% air. The plates were then centrifuged at 80g for 5 min at 24° C in a PR-J (IEC) centrifuge using rotor No. 259 (IEC) and cups No. 384 (IEC). The nonadherent cells were collected after a brief agitation, washed once in MEM, counted, and used for inoculation. Cell-mediated cytotoxicity was measured essentially as described before¹⁸: 0.1 ml containing 2.5 × 10⁴ ⁵¹Cr-EL-4 cells in MEM + 10% foetal calf serum was mixed with 0.2 ml containing the appropriate number of spleen lymphocytes to yield the desired effector to target cell ratio. The cell mixtures were incubated at 37° C in a humidified atmosphere of 5% CO₂ and 95% air for 3 h on a rocking platform. After incubation, 1 ml of medium was added, the cells were centrifuged at 400g for 10 min, and the supernatant was counted for radioactivity. The results are expressed in specific percentage lysis ± s.e. and represent the mean of tests run in triplicate. The percentage specific lysis was calculated as follows:

$$\frac{\text{Test counts—background counts}}{\text{Total releasable counts—background counts}} \times 100$$

where background counts were released in the presence of normal spleen and total releasable counts represent approximately 80% of total counts.

irradiated SJL/J mice injected with 4 × 10⁷ BALB/c splenocytes either unfractionated or depleted on C57BL/6 monolayer all died within 3 weeks (not shown).

At first, we thought that the fractionated BALB/c spleen cells used to inoculate the irradiated C57BL/6 mice could have been contaminated with C57BL/6 cells eluted off the monolayers during the adsorption procedure, so that the syngeneic C57BL/6 cells were actually responsible for protection and survival of the irradiated mice. This was ruled out as follows. Elution of C57BL/6 cells from the monolayers was estimated not to exceed 1.5 × 10⁶ cells, or roughly 10% of the BALB/c spleen cells recovered from each monolayer. Mice inoculated with a mixture of 5 × 10⁷ unadsorbed BALB/c cells and 5 × 10⁶ C57BL/6 spleen cells (Table 2, experiment 2) all died, as did control mice inoculated with 5 × 10⁷ unadsorbed BALB/c spleen cells. Moreover, BALB/c spleen cells fractionated on mitomycin C-treated (40 µg ml⁻¹ containing 5 × 10⁷ lymphocytes for 30 min at 37° C) C57BL/6 spleen cell monolayers could also reconstitute irradiated C57BL/6 mice.

The mice that survived the allogeneic transplant of fractionated cells were examined 30 d later for lymphoid cell chimaerism by complement-dependent cytotoxic assays using specific allogeneic antisera. The spleens were composed predomi-

Table 2 Survival of lethally irradiated C57BL/6 mice inoculated with fractionated allogeneic BALB/c spleen cells

Experiment	No. of BALB/c donor cells	Spleen cell monolayer used for adsorption	No. of mice alive* No. of mice used
1	None	None	0/10
	2×10^7	None	0/10
	4×10^7	None	0/10
	2×10^7	C57BL/6	5/9
	4×10^7	C56BL/6	7/10
	6×10^7	C57BL/6	3/4
2	5×10^7	None	0/5
	5×10^7	BALB/c	0/5
	5×10^7	SJL/J	1/6
	5×10^7 BALB/c +	None	0/5
	5×10^6 C57BL/6		
	5×10^7	C57BL/6	4/6

* These results were derived 120 d after inoculation. A few animals died 1–5 d after inoculation and were omitted from the group.

nantly of host lymphoid cells (Table 3). These results, however, were not unexpected, for spleen cells have been considered to be a poor source of stem cells. The exact mechanism by which the animals remain alive is not known. Donor allogeneic spleen cells, incapable of triggering a GvH reaction, may serve as transient cells and help the host to recover from the immediate effects of irradiation.

These experiments show that: first, fractionated allogeneic spleen cells inoculated into lethally irradiated recipients permit the host to recover from the combined fatal effects of irradiation and GvH disease; and second, a specific compartment of immunocompetent GvH reactive cells can be depleted from a normal spleen lymphoid cell preparation by adsorption on allogeneic cell monolayers. Previous failures to demonstrate

Table 3 Origin of spleen lymphoid cells obtained from irradiated C57BL/6 mice that survived allogeneic spleen cell transplants*

Treatment	% Dead cells†		
	Normal C57BL/6 spleen	Normal BALB/c spleen	Reconstituted spleen
Complement alone	15	15	15
Anti-BALB/c serum (1:10)	15	15	15
Anti-BALB/c serum (1:10) + complement (1:5)	15	85	20
Anti-C57BL/6 serum (1:10)	15	15	15
Anti-C57BL/6 serum (1:10) + complement (1:5)	70	15	65

* This group of mice was killed 30 d after inoculation of BALB/c spleen cells fractionated on C57BL/6 spleen cell monolayers.

† The values represent the percentage of lymphoid cells killed in a complement-mediated cytotoxic assay as determined by the trypan blue (0.2%) dye exclusion technique. Fresh rabbit serum was the source of complement after being absorbed with normal BALB/c and C57BL/6 spleen cells (2 volumes of serum + 1 volume of cells at 4°C for 1 h). Anti-BALB/c and anti-C57BL/6 sera were prepared in C57BL/6 and BALB/c mice, respectively, by three biweekly injections of 2×10^7 spleen cells. The spleen cells used in the cytotoxic assays were treated first with ammonium chloride (0.14 M) in Tris buffer to remove red blood cells. Spleen cells (3×10^5 , 0.1 ml) were incubated with 0.1 ml of antiserum for 30 min at 24°C, then 0.1 ml complement was added and the mixture was incubated for a further 30 min at 37°C.

depletion of T helper cell¹⁹ or of GvH reactive cells from sensitised²⁰ or nonsensitised²¹ cells may have been due to technical problems and not to the absence of antigen-binding receptors on the precursor cells. It seems likely that the centrifugation step used by us in the adsorption process allows firm binding of the receptor-bearing cells to the membrane antigens. Our results indicate that unsensitised precursor cells carry antigen-binding receptors on their surface, and agree with the findings of Lonai *et al.*¹³ and Altman *et al.*¹⁴. We believe that the techniques used in our study could be applied in bone marrow transplantation, immunodeficiency diseases, and the immunotherapy of cancer.

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Subunit complex III-IV as the nucleotide binding site of Q_B replicase

Of the four subunits of Q_B replicase, I, III and IV are bacterial proteins and II is coded for by the phage genome^{1,2}. Among host-derived subunits, I is identified as factor i, a protein which controls cistron-specific translation in *Escherichia coli*^{3,4}, and III and IV are identified as protein synthesis elongation factors, EFTu and EFTs (ref. 5), respectively.

Several lines of evidence indicate that EFTu and EFTu-EFTs interact with GDP or GTP (refs 5–8) and that EFTs stimulates the exchange of GDP bound to EFTu with unbound GTP (refs 9–12), but does not interact with these nucleotides. It has not been known whether EFTu and EFTs in the Q_B replicase perform functions analogous to those in protein synthesis.

Recent work in our laboratories (ref. 13 and unpublished results) has shown that GTP binding to EFTu-EFTs from *E. coli* HAK 88, a mutant with altered EFTs (ref. 14), is thermolabile and that this is also the case for Q_B replicase formed in infected cells. These results support the suggestion that subunit complex III-IV is concerned with nucleotide binding, or at least GTP binding.

I report here results concerning the nucleotide-binding site of Q_B replicase, which indicate that subunit complex III-IV can account for the ability of the enzyme to bind all four nucleotides, whereas I-II cannot, and that there are two distinct nucleotide-binding sites on the enzyme and its subunit

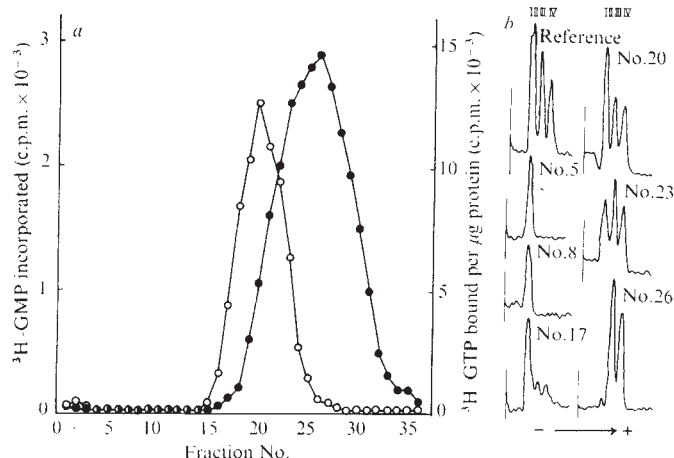


Fig. 1 Sedimentation pattern of Q β replicase subunits carrying enzyme activity and GTP binding ability. Q β replicase was purified as described previously¹⁶. The glycerol density gradient fraction with a specific activity of 5,500 U mg⁻¹ protein was used and revealed only four bands corresponding to the enzyme subunits I, II, III and IV^{1,2} using sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis¹⁷. Q β replicase (200 U) was dissociated into its subunit complexes I-II and III-IV by dialysis for 4 h at 0°C against a buffer containing 0.01M Tris HCl (pH 7.6), 1 mM Mg acetate, 2×10^{-4} M EDTA, 1×10^{-4} M dithiothreitol and 5% glycerol. The dialysate was centrifuged on 5 ml of 10–30% glycerol density gradient made up in the same buffer for 17 h at 37,000 r.p.m. at 6°C in the RPS-40 rotor of Hitachi ultracentrifuge 55P-2. A fraction (0.15 ml) was collected from the bottom of the tube and aliquots were used for analysis of enzyme components by SDS-polyacrylamide gel electrophoresis¹⁷, determination of protein concentration¹⁸, Q β replicase assay (○) with poly(C) as template¹⁶ and ^3H -GTP-binding (●). The reaction mixtures for the GTP-binding assay contained in 0.25 ml: 25 μmol Tris HCl (pH 7.6), 2.5 μmol MgCl₂, 1 μmol 2-mercaptoethanol, 0.22 μCi ^3H -GTP (12 Ci mmol⁻¹) and enzyme components from the gradient. After incubation for 20 min at 30°C, the reaction mixture was passed with gentle suction through a nitrocellulose membrane filter (Sartorius MF 50, 0.45 μm , 12 mm) presoaked in a buffer containing 0.1M Tris HCl (pH 7.6), 0.01M MgCl₂, 0.025M NaCl and 4×10^{-4} M 2-mercaptoethanol and washed with 0.25 ml of the same buffer. The SDS-polyacrylamide gels (acrylamide 7.5% bis-acrylamide 0.2%, SDS 0.1%, sodium phosphate 0.1M, pH 7.5) were run for 3 h at 7.5 mA per gel, stained with Coomassie brilliant blue and destained¹⁷. The gels were scanned at 600 nm in a Toyo DMU-2 scanner. *a*, Q β replicase and GTP-binding activities of fractions from the gradient; *b*, SDS-polyacrylamide gels of fractions from the gradient and the purified Q β replicase as reference.

complex III-IV, one of which is GTP-specific and the other is probably common to nucleotides other than GTP or to all four.

To examine the binding of nucleotides to Q β replicase and its subunit complexes I-II and III-IV, ^3H -nucleotide was incubated with protein, filtered through a nitrocellulose membrane filter and washed with a buffer as described in the legend to Fig. 1. ^3H -radioactivity bound to the membrane filter was then measured as described previously¹⁵. Q β replicase was dissociated into subunit complexes I-II and III-IV as described by Kamen². The two complexes were separated by centrifugation on a glycerol density gradient (Fig. 1). Subunit complex I-II sedimented in fractions 4 to 14 and subunit complex III-IV in fractions 25 and 26 of the gradient; replicase activity, assayed with poly(C) as template, appeared in fractions 17 to 24 with a main peak in fraction 20.

^3H -GTP binding was also determined in an aliquot of each fraction of the gradient. Essentially no ^3H binding was observed with fractions 2 to 14. Binding, which was first seen in fraction 16, reached a maximum in fraction 26 and declined in subsequent fractions. Fraction 26 contained the largest quantity of the III-IV complex. The amount of ^3H -GTP bound per μg of protein in fraction 20 was about 40% of that in

fraction 26. The molecular weight of Q β replicase and its subunit complex III-IV has been assumed to be 210,000 and 80,000 (refs 1, 2), respectively, and I found that the amount of ^3H -GTP bound per molecule of protein was about the same for these two components.

Binding of ^3H -GTP to Q β replicase and its subunit complexes I-II and III-IV was also compared using various concentrations of protein and constant GTP (Fig. 2). The amount of ^3H -GTP bound increased linearly with increasing quantities of protein when the replicase or its subunit complex III-IV was used. The amount of ^3H -GTP bound per mole of subunit complex III-IV was about 85% of the amount bound per mole of enzyme. On the contrary, there was no detectable binding of ^3H -GTP when up to 5 pmol of subunit complex I-II was added.

Since no detectable protein, including replicase and its subunit complexes I-II and III-IV, pass through a membrane filter even in the presence of nucleoside triphosphates, it seems unlikely that they form a nucleotide-protein complex which could pass through the membrane. Further, since Q β replicase activity was reconstituted from subunit complexes I-II and III-IV and from I-II and EFTu-EFTs of *E. coli* when incubated as described by Kamen², subunit complex I-II does not seem to be inactivated during preparation and storage. Thus it is concluded that, unlike Q β replicase and its subunit complex III-IV, subunit complex I-II cannot bind ^3H -GTP under the conditions used.

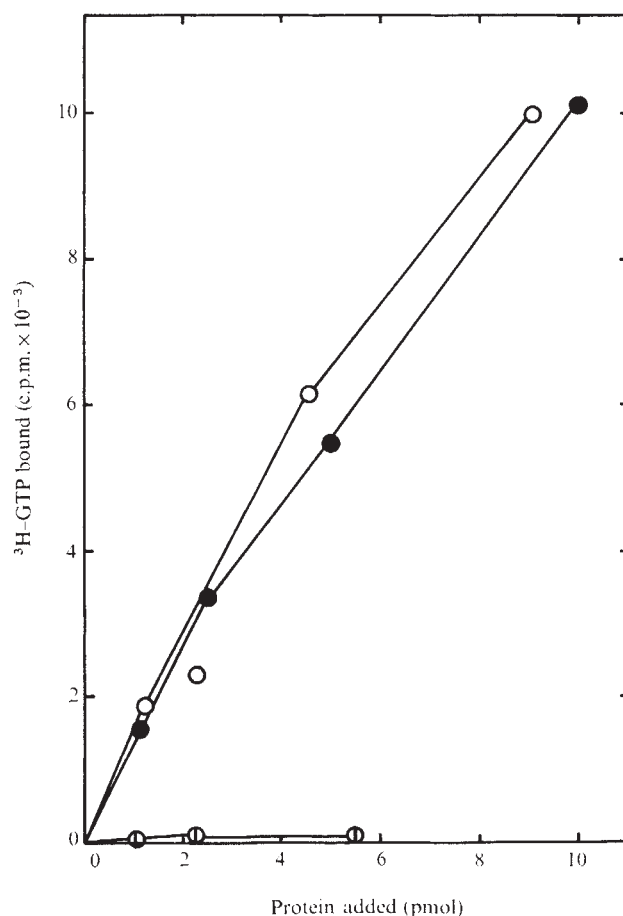


Fig. 2 Comparison of ^3H -GTP-binding ability of Q β replicase and its subunit complexes I-II and III-IV. The complexes were prepared as described in Fig. 1. ^3H -GTP-binding was carried out as described in Fig. 1 with various quantities of the replicase (○), subunit complex I-II (◐) or subunit complex III-IV (●) and 0.22 μCi of ^3H -GTP (12 Ci mmol⁻¹). The amount of protein added was calculated by assuming that the molecular weight of enzyme subunits I, II, III and IV are 70,000, 65,000, 45,000 and 35,000 (ref. 2), respectively, and that one molecule of enzyme, subunit complexes I-II and III-IV consist of one molecule each of their respective components.

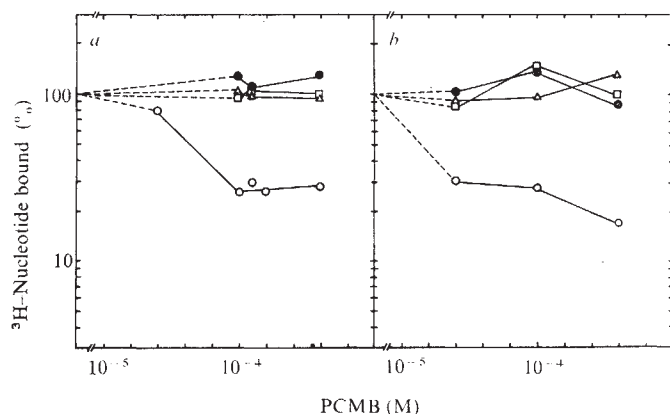


Fig. 3 The effect of PCMB on the binding of ^3H -nucleotides to Q_β replicase and its subunit complex III-IV. Q_β replicase and subunit complex III-IV, previously dialysed against 0.05M Tris HCl (pH 7.6) containing 0.01M MgCl_2 and 20% glycerol were used. The nucleotide-binding assay was carried out for 20 min at 25°C after preincubation of 4.5 pmol of the enzyme (a) or 2.5 pmol of subunit complex III-IV (b) with the indicated concentrations of PCMB for 10 min at 30°C . In these assays, 0.46 μCi of ^3H -ATP (33 Ci mmol^{-1}), 0.35 μCi of ^3H -CTP (30 Ci mmol^{-1}), 0.15 μCi of ^3H -GTP (12 Ci mmol^{-1}) or 0.49 μCi of ^3H -UTP (27 Ci mmol^{-1}) was added to each tube. The amount of ^3H -nucleotide bound was measured as described in Fig. 1 and is given as the percentage of the nucleotide bound to enzyme or subunit complex in the absence of PCMB. The amounts of ^3H -labelled ATP, CTP, GTP and UTP bound to the control enzyme were 2,558, 2,449, 5,404 and 2,388 c.p.m., respectively, and to the control subunit complex III-IV, 2,612, 1,643, 2,821 and 2,825 c.p.m., respectively. ●, ^3H -ATP; ▲, ^3H -CTP; ○, ^3H -GTP; □, ^3H -UTP.

To localise sites for other nucleotides, I have examined the binding of ^3H -ATP, CTP and UTP to these protein components. Each nucleotide reacted with subunit complex III-IV as well as with Q_β replicase (Table 1), although, in either case, bindings of ATP, CTP and UTP were about one order of magnitude weaker than that of GTP. By contrast, significant binding with subunit complex I-II was not observed by either nucleotide-binding assay. These results support the idea that all four nucleotide-binding sites of Q_β replicase are located in subunit complex III-IV rather than I-II. The nucleotide-binding of complex III-IV seems to be somewhat low compared

with those of Q_β replicase (Table 1), but can account for about 70% of those of the enzyme. Thus, it seems that subunit complex III-IV needs to interact with other subunit(s) in order to function normally.

In an attempt to obtain more information about the nucleotide binding site, the effect of the sulphhydryl blocking reagent, *p*-chloromercuribenzoate (PCMB), on nucleotide-binding to the enzyme was examined. PCMB inhibits the enzyme completely at a concentration of 1×10^{-5} M (ref. 19). About 70% of ^3H -GTP binding of the enzyme was blocked by 1×10^{-4} M PCMB, whereas the binding of ^3H -ATP, CTP and UTP to the enzyme was insensitive to the reagent even at a concentration of 6×10^{-4} M (Fig. 3a). A similar correlation was observed with the effect of PCMB on nucleotide-binding to subunit complex III-IV (Fig. 3b). These results strongly suggest that there are two different types of nucleotide-binding sites on the enzyme and its subunit complex III-IV; one of which is a PCMB-sensitive site which is of the GTP specific and the other, a PCMB-insensitive site. This possibility is supported in part by evidence that neither ATP, CTP nor UTP interfere with the binding of ^3H -GTP to subunit complex III-IV⁵. At present it is still obscure whether the second type of binding site, or PCMB-insensitive site, is common to nucleotides other than GTP and whether PCMB-sensitive GTP binding site is involved, throughout all the processes, in Q_β RNA replication.

Using Q_β replicase with a temperature-sensitive subunit IV, subunit IV has been found to be involved in an early stage of the RNA replication but not in RNA chain elongation when Q_β RNA is the template (my unpublished results). Furthermore, this protein was shown to stimulate specifically the binding of GTP to EFTu-GDP. It is more likely therefore that subunit III *per se* carries binding sites for all four ribonucleoside triphosphates, while subunit IV acts as an effector protein controlling the affinity of nucleotide-binding to the enzyme, particularly the binding of GTP, which is required for initiation of RNA synthesis²⁰. As Blumenthal *et al.*⁵ pointed out, it is possible that these components in Q_β replicase also take part in a specific interaction with RNA in order to initiate replication since subunit complex III-IV can form complex with Q_β RNA retainable on a membrane filter (unpublished observation).

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Table 1 Binding of ^3H -nucleotides to Q_β replicase and its subunit complexes I-II and III-IV

^3H -nucleotide added	pmol	^3H -nucleotides bound, pmol per pmol protein	I-II	III-IV
		I-II-III-IV		
^3H -GTP	2.6	0.05	<0.001	0.03
	5.2	0.09	<0.001	0.06
	13.2	0.18	0.001	0.16
	26.4	0.33	0.002	0.21
^3H -ATP	13.2	0.04	<0.001	0.03
	26.4	0.06	<0.001	0.05
	69.0	0.14	0.001	0.12
	139.0	0.29	0.001	0.21
^3H -CTP	57.9	0.10	<0.001	0.06
	116.0	0.17	<0.001	0.11
	174.0	0.27	0.001	0.17
^3H -UTP	17.9	0.04	<0.001	0.02
	89.0	0.16	<0.001	0.12
	179.0	0.36	0.002	0.21

Q_β replicase and its subunit complexes I-II and III-IV were prepared as described in Fig. 1. The binding assay was carried out for 20 min at 30°C as described in Fig. 1 with ^3H -ATP (33 Ci mmol^{-1}), ^3H -CTP (30 Ci mmol^{-1}), ^3H -GTP (12 Ci mmol^{-1}) or ^3H -UTP (27 Ci mmol^{-1}) in the presence of 4.5 pmol of Q_β replicase, 3.84 pmol of subunit complex I-II or 5.0 pmol of subunit complex III-IV. Protein concentration was calculated as described in Fig. 2. The purity of ^3H -nucleotides used was estimated to be 99% by paper chromatography.

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Effect of ATP on release of intracellular enzymes from damaged cells

THE use of serum enzyme measurements as aids to the diagnosis of disorders of the heart, liver and other organs is established practice in clinical laboratories everywhere, but little is known of the mechanisms whereby intracellular enzymes are discharged from damaged cells. In necrotic states, complete disruption of the cell will lead to release of its contents, but in reversible inflammatory states the cause of the increased membrane permeability remains obscure.

In an attempt to devise a model for the study of the factors affecting the liberation of intracellular enzymes, we recently described the effects of phospholipases A and C, on enzyme release in rat lymphocytes and human leukocytes¹. In these experiments the porosity of the cell membrane was increased leading to the efflux of cytosolic, and to a lesser extent mitochondrial, enzymes into the medium. We have now investigated the actions of a number of substances which might protect the cell against the effects of phospholipases as judged by a reduction in the leakage of intracellular lactate dehydrogenase. One of these was adenosine triphosphate, ATP, and its effects are described here.

Rat lymphocyte suspensions were prepared in Krebs-Ringer buffer solution (pH 7.4) containing glucose (8.3 mmol l⁻¹) and were subjected to phospholipase A as previously described¹. The experiments were carried out in flasks which were gently shaken in the water-bath (37° C) of a Warburg apparatus. In parallel experiments, ATP was

incorporated into the reaction medium 10 min before the addition of the phospholipase. Incubation at 37° C was continued for a further 30 min, after which samples were removed and centrifuged. Lactate dehydrogenase activities were determined in the supernatant and also in sonicated homogenates of the cell suspension to provide an estimate of the total activity. The spectrophotometric method of Wróblewski and LaDue² with pyruvate as substrate was used in 25° C.

The results shown in Fig. 1, indicate that at concentrations greater than 5 mmol l⁻¹, ATP reduces the amount of lactate dehydrogenase released from suspensions of rat lymphocytes as the result of the action of phospholipase A. No such effect was observed with ADP in similar concentrations. The protective effect was proportional to the concentration of ATP. In control preparations from which the phospholipase was omitted only small amounts of lactate dehydrogenase were released, and it was uncertain whether

Table 1 Effect of ATP on the release of lactate dehydrogenase from rat lymphocytes

ATP concentration (mmol l ⁻¹)	No. of experiments	Enzyme activity lost from cells (% of control)	
		Mean ± s.d.	Significance
5	7	83 ± 24.9	<i>P</i> < 0.05
10	7	74 ± 21.6	<i>P</i> < 0.005
50	7	32 ± 19.1	<i>P</i> < 0.001

Cells from different rats in each experiment were incubated for 3 h at 37° C in Krebs-Ringer solution (pH 7.4).

ATP exerted a protective effect in these also. We excluded the possibility that ATP had an inhibitory effect on lactate dehydrogenase by demonstrating comparable activities in homogenates containing and omitting ATP, and also by adding ATP to some of the samples during enzyme determination.

Similar effects were observed when the output of aspartate transaminase, malate dehydrogenase and glucose 6-phosphate dehydrogenase activities was measured.

A second series of experiments was carried out in which rat lymphocytes were incubated for 3 h at 37° C in the absence of phospholipase A. During this period the intracellular ATP concentration of a control preparation, determined by the method of Adam³, declined from 1.75 mmol l⁻¹ at 30 min to <0.01 mmol l⁻¹ after 3 h, and the release of lactate dehydrogenase increased from 15% to 67 ± s.d. 16%. Incorporation of ATP into the medium led to significant reductions in the enzyme activity lost from the cells (Table 1).

The protective effect ATP in physiologically relevant concentrations observed here is consistent with the view that maintenance of cell membrane integrity is associated in some way with the energy content of the cell. This indicates that any condition which leads to a diminution of intracellular ATP is likely to result in leakage of enzymes and other proteins. Such a relationship was suggested by Sweetin and Thomson⁴ to explain their observation that human erythrocytes did not release significant amounts of enzymes until the cells had metabolised all the glucose in the medium. Our work provides experimental evidence in support of this suggestion.

As well as acting as a reserve of energy, ATP may have a direct effect in maintaining the integrity of the cell membrane, as it is known to be concerned in the biosynthesis of phosphatidic acids⁵, intermediates in the synthesis of phospholipids, the main components of the membrane. The belief that ATP is almost exclusively an intracellular material and that it cannot enter the cell from extracellular fluids is difficult to reconcile with our findings. The protective action may be associated with a direct effect on the cell membrane or with the uptake of ATP from the medium. We point out that such a process has been observed in rat muscle by Chaudry and Gould⁶.

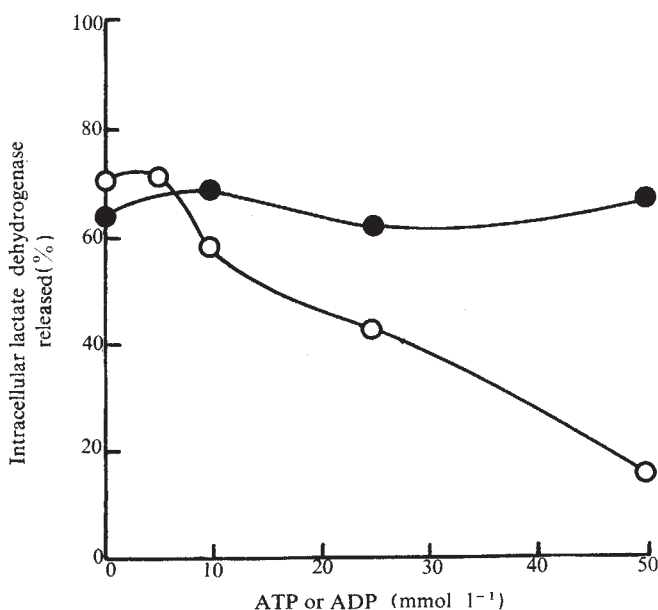


Fig. 1 Effect of ATP (O) and ADP (●) on the leakage of lactate dehydrogenase from rat lymphocytes evoked by phospholipase A (50 mU ml⁻¹).

The concept that intracellular enzyme release is secondary to reduction in the ATP content of the cell provides an explanation for the efflux of aldolase from intact rat muscles in response to anoxia, deprivation of glucose and exposure to high potassium ion concentrations reported by Zierler⁷. Glucose is the primary source of ATP synthesis, anoxia leads to accumulation of NADH and failure of the tricarboxylic acid cycle and thus to the inhibition of ATP synthesis, while the high potassium concentration interferes with the action of the Na^+/K^+ pump, the energy for which is provided by the hydrolysis of ATP by ATPase.

Discharge of intracellular enzymes into the circulation occurs in tissue damage due to a variety of causes, such as virus infection, trauma, chemical toxicity, hypoxia, invasion by a malignant process and so on. The suggestion that failure to synthesise ATP in adequate amounts may be a common factor seems to merit further investigation.

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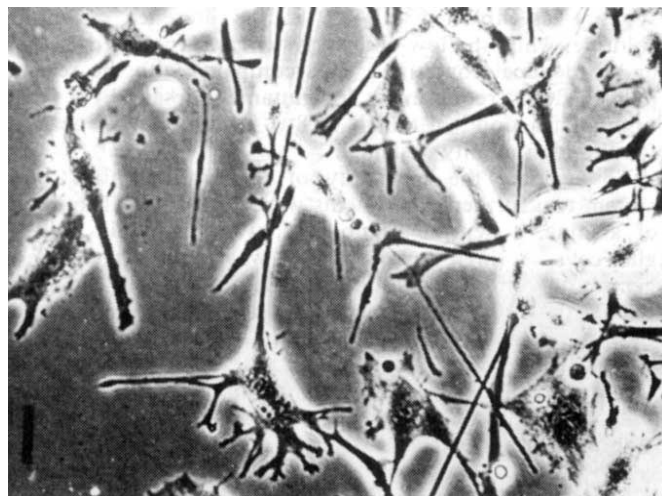


Fig. 1 Positive phase contrast photomicrograph of living rat C-6 glioma cells in normal growth medium 48 h after a 40 h pulse in 10^{-7} M amethopterin. Astroblastic cells soon begin to resemble normal astrocytes. The multipolar processes become very long and develop broad end feet. Bar represents 50 μm .

of cultured rat astrocytoma (given by Dr G. Sato), to accumulate glutamate. We consider that the use of both these preparations can most reliably suggest *in vivo* properties of glial cells: with bulk isolated glia alone there are problems due to cell damage and contamination, and with cultured cells dedifferentiation and the expression of anomalous metabolic properties can be troublesome.

Bulk isolated glial cell fractions were prepared as described before⁴ and were examined by electron microscopy and by the addition of labelled synaptosomes to the initial tissue suspension; both methods indicated less than 10% synaptosomal contamination in the glial fractions. The C-6 clonal line grows in our hands as morphologically differentiated cells, with multiple processes. A 24 h exposure to amethopterin (10^{-7} M) during the third day of logarithmic growth, however, produced cells which appeared to be even further morphologically differentiated (Fig. 1). In addition, the glia-specific protein S-100 increased after amethopterin treatment⁹ further indicating that these cells can be induced to express the properties of differentiated glial cells.

Uptake experiments were carried out as described before³. Under the conditions used less than 10% of the amino acid is metabolised when examined by paper chromatography. Samples were oxidised in a Packard oxidiser and counted in a Packard liquid scintillation counter. The results are expressed as nmol of substrate accumulated per mg dry weight in 4 min.

The uptake of glutamate by cultured glial cells is illustrated in Fig. 2. Table 1 shows that these results, including the demonstration of high and low affinity uptake systems, correspond remarkably to values reported for the uptake of glutamate by synaptosomal preparations. As a further check on the correspondence between the ability of the synaptosomal fraction to accumulate glutamate and that of the cultured glial cell, the Na^+ dependence of glutamate accumulation was investigated. Solutions containing sucrose or choline chloride were substituted for sodium chloride solutions. The results indicate that the high affinity uptake

Uptake of the neurotransmitter candidate glutamate by glia

GLUTAMIC acid is the primary candidate for a role as an excitatory transmitter at synapses mediating the primary afferent pathway and cortical pathways in mammals¹. Seeking to gain evidence for this, various laboratories have looked for a mechanism for the efficient removal of glutamic acid from the synaptic cleft. Following the paradigm of monoamine synapses, in which high affinity uptake systems apparently localised in the presynaptic membrane remove transmitter after its action at the postsynaptic membrane², high affinity uptake systems for glutamate removal have been sought. Logan and Snyder³ described a high affinity transport system for glutamate in a synaptosomal preparation, and postulated that it might serve to localise glutamate-mediated synapses. Recent evidence has suggested that the transport systems involved in the removal of putative amino acid transmitters from the synaptic cleft are not localised exclusively in the synapse.

Bulk isolated glial cells have a high affinity uptake system for γ -aminobutyrate (GABA)⁴ that seems suitable for participation in the removal of GABA from the synaptic cleft. Autoradiography of a lobster nerve-muscle⁵ preparation and rat retina⁶ also suggests that GABA is accumulated by glial cells. Similar studies⁷ implicated glia in the removal of glycine in spinal cord preparations, glutamate in cortical areas and GABA in the cerebellum.

We have investigated further the ability of glial cells, either obtained as bulk cell fractions or from the C-6 line

Table 1 K_m values for glutamate uptake

Bulk isolated glial cells	$1.2 \pm 0.4 \times 10^{-5}$	M high affinity
	$1.3 \pm 0.7 \times 10^{-4}$	M low affinity
Cultured glial cells	$6.6 \pm 1.2 \times 10^{-5}$	M high affinity
	$1.3 \pm 0.8 \times 10^{-3}$	M low affinity
Synaptosomal fraction ³	$3.6 \pm 1.3 \times 10^{-5}$	M high affinity
	$1.5 \pm 0.9 \times 10^{-3}$	M low affinity

system was inhibited $81 \pm 4\%$ in the presence of choline chloride, while the low affinity system was inhibited $22 \pm 3\%$ under identical conditions. This compares with the recently published Na^+ requirement for glutamate uptake by a synaptosomal fraction¹⁰ in which 95.8% inhibition was obtained for the high affinity system.

To evaluate the specificity of the high affinity uptake system two further experiments were performed. The uptake of alanine, a neutral amino acid, was studied in cultured glial cells. A single uptake system was found over the concentration range of 10^{-3} to 10^{-7} M. This system had a K_m of $1.0 \pm 0.5 \times 10^{-3}$ M. A line of cultured HeLa cells exhibited only a single uptake system for glutamate, with a K_m of $3.8 \pm 1.3 \times 10^{-3}$ M. This suggests that high affinity uptake systems are not involved in the accumulation of all amino acids by glia, and that glutamate uptake mediated by high affinity transport systems is not a general property of other cell types.

To investigate the possibility that glial elements contribute significantly to glutamate uptake by the synaptosomal fraction, it is necessary to show that glia can form small vesicles which can accumulate glutamate. Following a report by Cotman *et al.*¹¹, we attempted to isolate a gliosome fraction from a sample of cultured cells using the standard Whittaker¹² procedure for isolation of synaptosomes. These

neuromuscular junction of arthropods. This synapse is cells, a glial element surrounding the synapse. Our demonstration of a high affinity uptake system for glutamate localised in glial cells and the findings of a similar system for GABA in glial cells together with autoradiographic evidence for glial accumulation of these compounds⁷ strongly suggests that if these amino acids are neurotransmitters, perisynaptic glial elements are involved in their inactivation.

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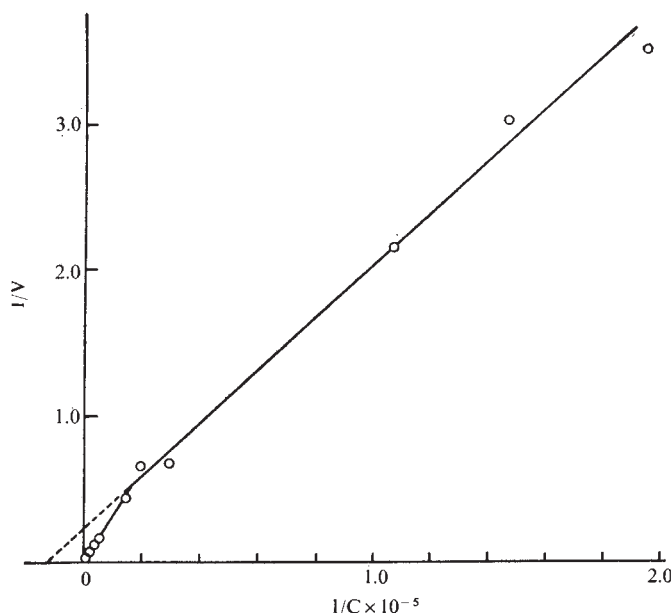


Fig. 2 A double reciprocal plot of the velocity of L-glutamic acid accumulation into glia from the C-6 line of rat glioma at 37°C and varying glutamic acid concentrations. The uptake media contained 140 mM NaCl, 5 mM KCl, 2 mM MgCl_2 , 3 mM CaCl_2 , 10 mM dextrose and 10 mM Tris HCl at pH 7.4. High affinity $K_m = 6.7 \times 10^{-5} \pm 1.3$; low affinity $K_m = 1.3 \times 10^{-3} \pm 0.8$.

gliasomes were incubated with 10^{-8} M glutamate and achieved tissue to medium ratios consistently in excess of 24 in 4 min. This uptake was temperature-sensitive and seemed to correspond to the high affinity uptake system characterised in intact cultured cells. This suggests that material isolated by the Whittaker procedure may contain appreciable non-neuronal elements.

Our results indicate that mammalian glial cells, prepared either as a bulk isolated fraction or from the C-6 rat glioma cell culture, have a high affinity transport system for the removal of extracellular glutamate. Glia have also been found to be involved in the uptake of glutamate by the neuromuscular junction of arthropods. This synapse is almost certainly mediated by glutamate¹³ and Feeder and Salpeter¹⁴ showed that most labelled cells in an arthropod

Involvement of brain cholinergic mechanisms in the action of chlorpromazine

THE function of cholinergic neurones in ganglia¹, striatum and other brain areas is normally regulated by dopamine (DA)² which, applied microiontophoretically, reduces the increased activity of caudate neurones elicited by acetylcholine³. Moreover, DA iontophoresis on caudate neurones mimics the responses elicited by electrical stimulation of the substantia nigra^{4,5}.

Chlorpromazine and haloperidol, neuroleptics which increase the turnover rate of DA in striatum^{6,7}, presumably block DA receptor function⁸. Such an action has been credited as an explanation for the antagonism by chlorpromazine of certain behavioural effects induced by amphetamine⁹. It is pertinent to remember that these amphetamine effects are thought to be mediated through the release of transmitter from dopaminergic neurones¹⁰.

Table 1 Turnover rate of ACL in rat striatum after haloperidol and chlorpromazine

Treatment ($\mu\text{mol kg}^{-1}$ i.p.)	ACh (nmol g^{-1})	Choline (nmol g^{-1})	ACh (d.p.m. nmol^{-1})	Choline (d.p.m. nmol^{-1})	ACh turnover rate ($\mu\text{mol g}^{-1} \text{h}^{-1}$)
Saline	48 $\pm$ 3.2	39 $\pm$ 2.8	228 $\pm$ 20	286 $\pm$ 33	0.98 $\pm$ 0.091
Haloperidol (5.2)	45 $\pm$ 4.1	42 $\pm$ 3.1	296 $\pm$ 32	299 $\pm$ 25	1.2 $\pm$ 0.10
Haloperidol (10.4)	47 $\pm$ 3.7	35 $\pm$ 1.1	330 $\pm$ 19*	227 $\pm$ 17	1.8 $\pm$ 0.15*
Chlorpromazine (9.3)	41 $\pm$ 6.0	37 $\pm$ 2.7	260 $\pm$ 23	312 $\pm$ 34	1.1 $\pm$ 0.06
Chlorpromazine (18.6)	50 $\pm$ 4.1	41 $\pm$ 4.9	370 $\pm$ 17*	285 $\pm$ 30	1.4 $\pm$ 0.10*

Phosphorylcholine-methyl- ^{14}C was infused intravenously at a constant rate for 6 min ($30 \mu\text{Ci kg}^{-1} \text{min}^{-1}$). Drugs were injected 1 h before beginning the infusion. The figures represent the mean values of four experiments $\pm$ s.e.m.

* $P < 0.05$ when compared with saline-treated animals.

We have investigated whether the action of chlorpromazine or haloperidol is associated with a change in the activity of brain cholinergic neurones. Since the amount of transmitter synthesised per unit of time is believed to be a good index of its *in vivo* utilisation¹¹ and since transmitter utilisation may change with neuronal activity¹², we have decided to monitor the effect of chlorpromazine and haloperidol on the turnover rate of acetylcholine (ACh) in occipital cortex and striatum of rats to assess whether when DA receptors are blocked, the synthesis of ACh in various areas of rat brain is increased. We have measured ACh turnover rate in these two brain structures because cholinergic neurones are present in various brain areas and dopaminergic endings are present in neocortex, limbic system, hypothalamic nuclei and striatum^{13,14}. However, in striatum a link between the two neuronal systems is suggested by the present understanding of the role of DA in Parkinson's disease.

partment of ACh in the same area and k_A , k_B and k_C denote the fractional rate constants of A, B and C, respectively. To measure k_C , which is the percentage of ACh pool metabolised per unit of time, and at steady state is equal to the rate of synthesis, the ratio of the specific activity of ACh and choline was calculated at 6 min of infusion and this value was multiplied by the concentration of ACh (in $\mu\text{mol g}^{-1}$). This value expressed by a given time unit gives an estimate of the turnover rate of Ch.

Tables 1 and 2 show the specific activities of ACh and choline in the striatum and occipital cortex of rats receiving saline, chlorpromazine or haloperidol 1 h before the constant rate infusion of radioactive phosphorylcholine for 6 min. Steady state concentrations of ACh and choline were not changed by the infusion of phosphorylcholine. Moreover, the choline concentrations in the two brain areas studied were comparable whereas the ACh concentrations in

Table 2 Turnover rate of ACL in rat occipital cortex after haloperidol and chlorpromazine

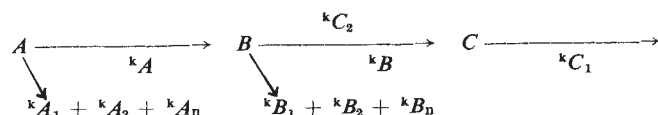
Treatment ($\mu\text{mol kg}^{-1}$ i.p.)	ACh (nmol g^{-1})	Choline (nmol g^{-1})	ACh (d.p.m. nmol^{-1})	Choline (d.p.m. nmol^{-1})	ACh turnover rate ($\mu\text{mol g}^{-1} \text{h}^{-1}$)
Saline	15 $\pm$ 1.1	30 $\pm$ 2.1	522 $\pm$ 53	1186 $\pm$ 120	0.14 $\pm$ 0.014
Haloperidol (5.2)	14 $\pm$ 2.1	29 $\pm$ 3.2	570 $\pm$ 69	1140 $\pm$ 104	0.15 $\pm$ 0.012
Haloperidol (10.4)	14 $\pm$ 1.8	33 $\pm$ 1.4	500 $\pm$ 73	1094 $\pm$ 81	0.14 $\pm$ 0.011
Chlorpromazine (9.3)	13 $\pm$ 2.6	37 $\pm$ 3.9	504 $\pm$ 80	1154 $\pm$ 137	0.15 $\pm$ 0.014
Chlorpromazine (18.6)	16 $\pm$ 1.2	35 $\pm$ 4.5	474 $\pm$ 78	1206 $\pm$ 97	0.14 $\pm$ 0.012

Phosphorylcholine-methyl- ^{14}C was infused intravenously at a constant rate for 6 min ($30 \mu\text{Ci kg}^{-1} \text{min}^{-1}$). Drugs were injected 1 h before beginning the infusion.

The figures represent the mean values of four experiments $\pm$ s.e.m.

Sprague-Dawley male rats (125–150 g) were infused at a constant rate with $0.65 \mu\text{mol kg}^{-1} \text{min}^{-1}$ of phosphorylcholine-methyl- ^{14}C ($44.0 \text{ mCi mmol}^{-1}$; New England Nuclear Co.) and killed at various times afterwards by microwave radiation focused on the skull (75 mW cm^2). The total power delivered down the wave guide was 1.5 kW at a frequency of 2.45 GHz. We found that when the heads of the rats were exposed to this radiation for 2–3 s, the brain choline and ACh concentrations were stabilised. The brain was dissected and processed for the assay of choline and ACh specific radioactivity as described by Hanin *et al.*¹⁵. As Fig. 1 shows, the specific activity of striatal ACh and choline increased with infusion time, and the rate of increase proceeded parallel with infusion time in both compounds. The percentage of the ACh store that was renewed per unit of time (fractional rate constant) was estimated at various times of infusion by the finite difference method (ref. 16 and Fig. 1). The fractional rate constant (k) multiplied by the steady state concentration of brain ACh yields an estimation of the amount of ACh made per unit of time.

Brain ACh turnover rate was calculated according to a method described in detail elsewhere¹⁵. In brief, this method assumes the following kinetic model



where A is the plasma choline compartment, B is the choline compartment in the brain area considered, C is the com-

partment of ACh in the same area and k_A , k_B and k_C denote the fractional rate constants of A, B and C, respectively. To measure k_C , which is the percentage of ACh pool metabolised per unit of time, and at steady state is equal to the rate of synthesis, the ratio of the specific activity of ACh and choline was calculated at 6 min of infusion and this value was multiplied by the concentration of ACh (in $\mu\text{mol g}^{-1}$). This value expressed by a given time unit gives an estimate of the turnover rate of Ch.

Tables 1 and 2 show the specific activities of ACh and choline in the striatum and occipital cortex of rats receiving saline, chlorpromazine or haloperidol 1 h before the constant rate infusion of radioactive phosphorylcholine for 6 min. Steady state concentrations of ACh and choline were not changed by the infusion of phosphorylcholine. Moreover, the choline concentrations in the two brain areas studied were comparable whereas the ACh concentrations in

striatum were three times larger than in occipital cortex. The specific activity of choline in cortex was three times that in striatum, suggesting that each brain area includes several choline compartments. The greater specific activities of choline in cortex than in striatum suggests that the choline compartment that is in rapid equilibrium with blood-borne choline in cortex is smaller than that of striatum. The data presented in the tables also suggest that the brain choline pool that is in rapid equilibrium with the plasma pool is that involved in the synthesis of ACh, because the specific activities of ACh and choline were greater in cortex than in striatum. The amount of ACh synthesised per unit of time was greater in striatum than in cortex: this difference exceeded the three-fold difference in pool size present in these tissues. The amount of ACh synthesised in striatum of rats receiving chlorpromazine ($18.6 \mu\text{mol kg}^{-1}$ intraperitoneally) or haloperidol ($10.4 \mu\text{mol kg}^{-1}$ intraperitoneally) 1 h before the infusion of phosphorylcholine was increased; there was no similar increase in the occipital cortex. These findings minimise the possibility that nonspecific effects of the two drugs are operative in explaining the increase of ACh turnover rate; actually, they suggest that these two drugs affect some neuronal mechanism that is important in the control of striatal ACh synthesis and less important in the control of the synthesis of ACh in occipital cortex. Since these two neuroleptics block striatal dopaminergic receptors⁸ and since DA concentrations in striatum are very high¹³ the action of haloperidol and chlorpromazine on dopaminergic and cholinergic mechanisms of striatum might be interrelated. A blockade of striatal DA receptors in striatum may account

for the extrapyramidal side effects evoked by the two drugs in several animals species, including man. This possibility is supported by reports that haloperidol is more potent than chlorpromazine in eliciting extrapyramidal side effects¹⁷ and in increasing striatal DA turnover rate (A. Revuelta, personal communication). The finding that chlorpromazine increases the rate of synthesis of brain ACh is in keeping with a report by Stadler *et al.*¹⁸ showing that chlorpromazine increases the release of ACh from the perfused striatum of rats. Chlorpromazine is credited for exciting an inhibitory action on cholinergic receptors when given in large doses.

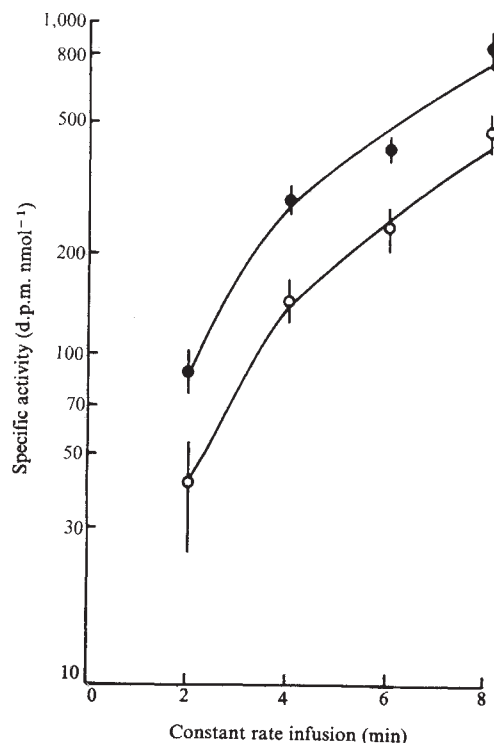


Fig. 1 ACh (○) and choline (●) specific radioactivities in rat striatum after phosphorylcholine(methyl-¹⁴C) (30 μ Ci kg⁻¹ min⁻¹) was infused for various minutes and the rats were killed immediately after microwave irradiation.

$$*k_{ACh} = 2(S_{ACh_{t2}} - S_{ACh_{t1}})/\Delta t(S_{Ch_{t1}} - S_{ACh_{t1}} + S_{Ch_{t2}} - S_{ACh_{t2}})$$

Δ min	* k_{ACh}
3-2	0.44
4-3	0.42
5-4	0.34
6-5	0.32
7-6	0.31
8-7	0.32

Such an action cannot explain the increased synthesis of striatal ACh elicited by chlorpromazine because haloperidol, which is more active than chlorpromazine in stimulating the synthesis of ACh, is less active in blocking cholinergic receptors¹⁷. Similar considerations can be made to exclude the fact that the anticholinesterase action of chlorpromazine is involved in the stimulation of striatal ACh synthesis¹⁷. In interpreting the physiological implications of the increase of ACh synthesis rate reported in the Tables, it would be important to know whether an increase of striatal ACh synthesis corresponds with an increase in the activity of cholinergic neurones. A direct answer to this question cannot be given at this time because the correlations between ACh turnover rate and the firing rate of cholinergic neurones has not been studied directly. Presumptive indirect evidence, however, supports the belief that the rate of ACh synthesis reflects the activity of cholinergic neurones (D. Cheney and M. Trabucchi, unpublished work).

Finally, it would be interesting to establish whether this increase of ACh synthesis in striatal cholinergic neurones is required to elicit an antipsychotic action. We are currently working to explore several factors involved. We found that clozapine, a neuroleptic with antipsychotic activity comparable with chlorpromazine but lacking the extrapyramidal side effects¹⁹, fail to increase the rate of ACh synthesis in striatum. Although, the present level of understanding would indicate that the increased biosynthesis of striatal ACh relates to the extrapyramidal side effects of neuroleptics rather than to their antipsychotic properties, more work is needed to reach a definite conclusion on this point.

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Effect of histamine H₂-receptor blockade on fasting serum gastrin and fasting gastric acid secretion in the dog

HISTAMINE receptors which are blocked by conventional antihistamines, such as mepyramine maleate, have been termed H₁-receptors. Receptors which are not blocked by these drugs have been found in the cardiac atrium, the uterus and the gastric parietal cell¹ and have been termed H₂-receptors. An antagonist of these receptors (burimamide) has recently been discovered². Histamine H₂-receptor antagonists inhibit gastric acid secretion stimulated by

histamine, pentagastrin, hypoglycaemia and food in the dog (M. I. Grossman and S. J. Konturek, unpublished) and by histamine and pentagastrin in cat (S. J. Konturek, unpublished), pig (G.O.B., M. W. Waterworth, M. D., and S. B., unpublished), and man³. Studies have shown that some hormonal inhibitors of gastric acid secretion, namely secretin⁴ and glucagon^{5,6}, depress fasting serum gastrin levels in man and a common mechanism of action through suppression of gastrin-mediated acid release has been postulated. This study attempts to determine whether burimamide has any effect on gastric acid secretion and gastrin release in basal conditions in the dog.

Two mongrel dogs weighing 17 and 25 kg were prepared with gastric fistulae, and subsequent studies were performed at least 3 months after surgery. Not more than one experiment was carried out every 48 h, and dogs were fasted for 16 h before each study. Gastric juice was collected every 15 min for 1.25 h after gastric collections had reached basal levels. After the first 15 min injections lasting 60 s of either burimamide (8 mg per kg body weight in a solution containing 15 mg ml⁻¹) or 0.15 M NaCl ml⁻¹ were given into a leg vein. In both dogs burimamide injections

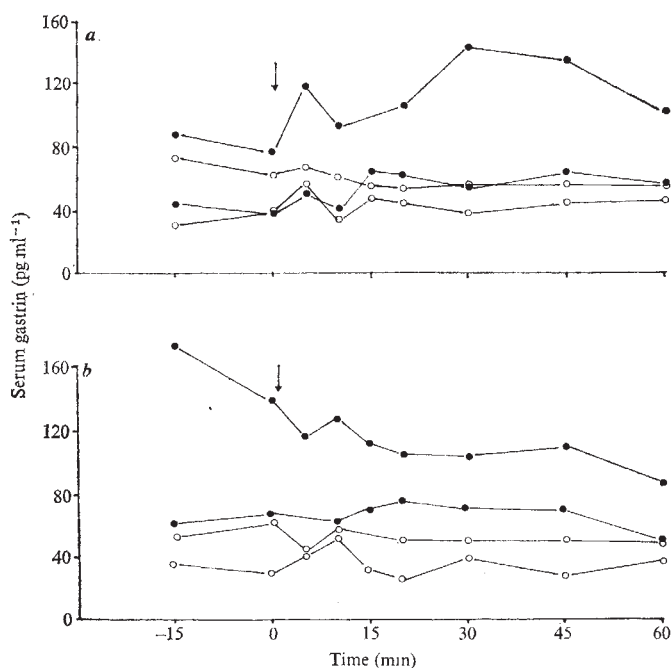


Fig. 1 Serum gastrin concentration in pg ml⁻¹. Burimamide (a) or saline (b) was injected at time 0. Open or closed circles denote studies performed in the same animals.

were followed by retching and restlessness which subsided within 2–5 min. Blood was taken from a separate leg vein 15 min before and 0, 5, 10, 15, 20, 30, 45 and 60 min after test injections. The blood was allowed to clot, and the serum separated and stored at -20° C. Gastrin was estimated later by radioimmunoassay (A. V. and B. G., unpublished). Gastric juice was measured to the nearest 0.5 ml and the acid titrated against 0.2 M NaOH using an automatic titrator (Radiometer, Copenhagen). Two burimamide and two control studies were performed in each dog in random order.

Figure 1 shows the serum gastrin concentrations in the four saline and the four burimamide-treated animals. In three test and four control studies there was minimal variation in serum gastrin concentrations from basal levels. A slight rise in serum gastrin levels occurred in only one test following burimamide. The differences between control and burimamide tests were not significant.

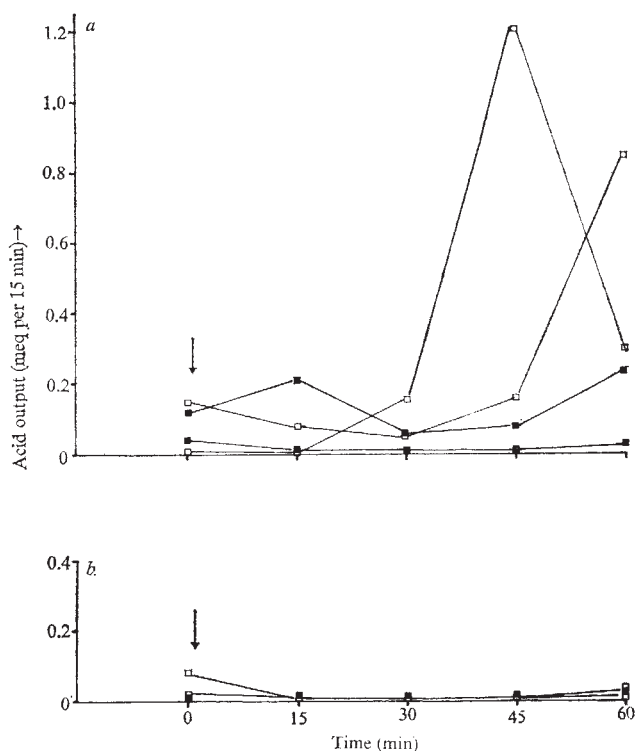


Fig. 2 Acid output from gastric fistulae in meq per 15 min. Burimamide (a) or saline (b) was injected at time 0. Open or closed squares denote studies performed in the same animal.

Two of the four dogs studied showed a slight rise in gastric acid secretion (Fig. 2) from 30–60 min after burimamide. The rise was, however, small in terms of output of acid, reaching a maximum of 1.20 and 0.72 meq per 15 min over basal levels in the two dogs. Dogs receiving control saline injections showed no increase in acid output.

The results of this study show that serum gastrin levels are not altered by burimamide given intravenously to fasting dogs. The reduction in acid secretion observed with burimamide is unlikely to be a consequence of lower levels of circulating gastrin. The slight rise in serum gastrin after burimamide in one study may be a secondary phenomenon following a slight reduction in gastric acidity and deinhibition of the antrum.

In searching for a unifying mechanism linking gastric acid secretion and gastrin release, earlier studies demonstrated that secretin⁷ and glucagon⁸ both inhibit gastric acid secretion, and they both depress fasting serum gastrin levels^{4–6}. In this respect they differ from H₂-receptor antagonists. Differences between the action of secretin and burimamide are amplified by studies on histidine decarboxylase in rat gastric parietal cells. Whereas secretin does not stimulate histidine decarboxylase in the rat⁹ recent studies have shown that both burimamide and metiamide produce a 3–5-fold rise in histidine decarboxylase levels in rat glandular stomach¹⁰. The compound SC 15396, an anti-gastrin, also produces a rise in histidine decarboxylase in the rat^{11,12} while inhibiting gastric acid secretion stimulated by both histamine and gastrin¹². It is thought that this antagastrin acts at the gastrin receptor site on the parietal cell¹¹. There is no evidence to support the concept that H₂-receptor antagonists inhibit gastric acid secretion by acting outside the parietal cell. It is, however, possible that histamine and gastrin act on different receptors on the same cell as postulated by Grossman and Konturek.

The late rise in gastric acid secretion following burimamide in two studies, although small in terms of milliequivalents of acid, was clearly not seen after the control

injections. Since the duration of action of burimamide given intravenously is short in both dogs (M. I. Grossman and S. J. Konturek, unpublished) and pig (G.O.B., M. W. Waterworth, M.D., and S.B., unpublished), the late rise in acid release may be the result of 'rebound' following withdrawal of the inhibitor. This phenomenon is known after certain calcium containing antacids¹³, and it would be clinically relevant if this phenomenon occurs when H₂-receptor antagonists are used therapeutically in man.

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Cone mechanisms initiating response of on-off goldfish optic fibres

ON-OFF ganglion cells are common in a wide variety of vertebrate retinæ¹, including those of primates (F. M. de Monasterio and P. Gouras, personal communication). These units respond with a short, high-frequency burst of action potentials when a stationary light is turned on in the receptive field and also when the light is turned off. Because a dark or light spot moving across the receptive field elicits a vigorous response, Maturana *et al.*² called on-off units in the frog "moving contrast detectors".

I have explored the properties of on-off fibres in the goldfish optic nerve. The generation of the on-off response in goldfish is of special interest because the goldfish retina has three classes of cones with peak sensitivity at 620, 530 and 455 nm (ref. 3), and the majority of ganglion cells give a colour-coded response⁴. I shall show that on-off fibres are a separate class of fibres with properties quite different from those of colour-coded units. I have determined the contribution of each of the three cone mechanisms in initiating the on and off components of the on-off response.

Common goldfish, *Carassius auratus*, were anaesthetised with tricaine during surgical exposure of the optic nerve, and were then immobilised with curare for the duration of the experiment. Tungsten microelectrodes coated with Insl-x were inserted into the optic nerve and the activity picked up from single fibres was amplified, displayed and tape recorded. The optical stimulation of the eye has been described in detail elsewhere⁵. The left eye of the fish was under water and at the centre of a 24-inch diameter plastic hemisphere filled with water. Stimuli were presented on a back-projection screen taped to the surface of the hemisphere being observed by the fish. The stimuli and coloured backgrounds which were projected on to the back-projection screen originated in high intensity Prado projectors fitted with electronic shutters and frames for holding neutral density filters, narrow band interference filters and interference cutoff filters.

Results were obtained for 16 on-off fibres in a total sample of 73 fibres. This is a higher percentage (22%) of on-off units than

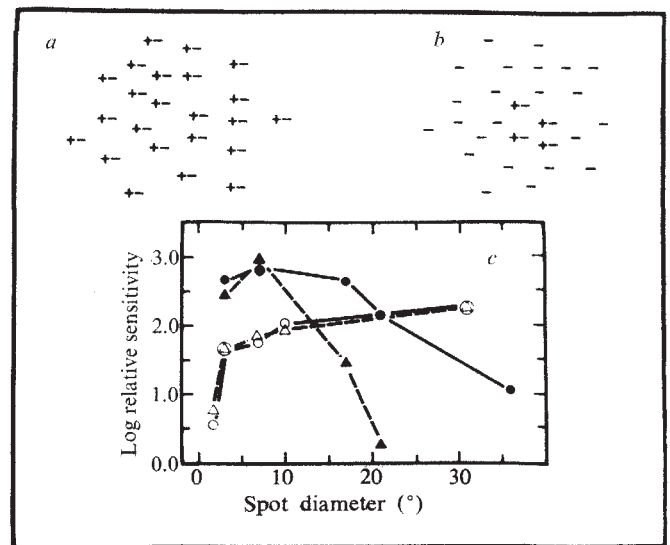


Fig. 1 Receptive field characteristics of on-off fibres. *a*, On-off receptive field centre having both on and off responses across the centre. The centre, about 20° in diameter, was mapped with a 1° spot 2.4 log foot-lambert in brightness. *b*, On-off receptive field centre having both on and off components at the midpoint, but only the off component towards the periphery. The width of the receptive field was about 10° mapped with a 2°, 1.8 log foot-lambert spot. *c*, Influence of spot size on sensitivity of the on (●○) and off (▲, △) components of the response. ▲, ●, An on-off fibre having an inhibitory surround which, for spot sizes above 10° in diameter depressed the sensitivity of both on (●) and off (▲) centre components. ○, △, An on-off unit which showed no reduction in sensitivity of the on (○) or off (△) centre response with larger spot sizes.

has been reported in a different preparation, the isolated goldfish retina (6%) (ref. 4). I established that both the on and off responses originated in a single optic fibre by showing that photographed action potentials in the on and off bursts had the same height and waveform.

Since stimulation of colour-coded receptive fields can result in an on-off response under certain conditions, I explored the receptive field of on-off units with stationary small spots of white light 1° or less in diameter. Neither the on nor the off components of the response were isolated in a central region of the receptive field. Either both on and off responses were present across the receptive field (Fig. 1*a*), or one or the other of the components dropped out at more peripheral receptive field positions (Fig. 1*b*). Both types of receptive field were quite different from the centre-surround, double-opponent organisation of colour-coded units⁴. The units described here were not directionally sensitive, a feature often associated with the on-off response in other animals. In goldfish optic nerve directionally sensitive fibres are present in small numbers⁵.

Area-threshold curves confirmed a previous finding in the frog⁶ that the excitatory on-off centre may have around it an inhibitory surround which raises the threshold for on and off components of the centre response (Fig. 1c, closed circles). In some cases, an inhibitory surround was not present (Fig. 1c, open circles). No response has been noted following stimulation of the surround alone. Within the on-off central region, there was an increase in sensitivity with larger stimulus sizes.

The cone mechanisms responsible for the on and off components were identified by making spectral sensitivity measurements. An example of the results is shown in Fig. 2. On-off responses were obtained to all monochromatic stimuli of 1 s duration at wavelengths from 400–720 nm. The thresholds for the on and off components were similar in this unit, although this was not always the case. Against a white background (Fig. 2a), both the on and off thresholds for responses above 500 nm fitted the retinene-2 nomogram of the red-absorbing goldfish pigment peaking at 620 nm. For 500 nm stimuli and below, the thresholds fell on the retinene-2 nomogram for the blue-absorbing cone pigment with peak sensitivity at 455 nm. A green mechanism on-off response was not evident against a white background but became apparent against a magenta background which reduced the red and blue mechanism sensitivities (Fig. 2b). The on and off thresholds against the magenta background for wavelengths between 500 and 620 nm fell on the retinene-2 nomogram for the green-absorbing cone pigment peaking at 530 nm.

Figure 2 shows that both the on and off response at a given wavelength are elicited by the same set of cone receptors, sensitive to red, green or blue. In the goldfish this was by no means a foregone conclusion, since on and off could have been elicited

by different cone receptor types. As is true with colour-coded goldfish optic fibres⁶, a single on-off optic fibre may receive connections from all three receptor systems. Only a few examples have been noted where one of the three cone systems could not be identified with the use of coloured adapting backgrounds. The strong blue mechanism response illustrated in Fig. 2 is characteristic of many optic fibres recorded from the intact, curarised goldfish⁶. The finding is somewhat puzzling in view of the failure of experiments using the isolated goldfish retina to see a blue mechanism response^{4,7} and of Mark's report³ that less than 7% of a sample of 113 goldfish cones contained blue-absorbing pigment. The spectral sensitivity curves of on-off fibres differ from those of colour-coded units in that there is no evidence of opponent interaction between red and green mechanisms. In colour-coded units this interaction tends to narrow opposing shoulders of the red and green mechanism spectral sensitivity curves at intermediate wavelengths where both red green mechanisms are stimulated simultaneously⁴.

I examined the latency and number of spikes for the on and off components of the responses as a function of intensity. For all wavelengths, and hence for all three cone mechanisms, there was a decrease in the latency of both on and off responses as intensity increased in 0.5 log unit steps above threshold. Occasionally, at the highest intensities, the off threshold would show a slight increase in latency. In general, the number of spikes in the on and off burst increased only slightly or not at all with increase in the stimulus intensity. This feature of the response indicates that on-off units would not have the dynamic frequency range necessary of a separate brightness channel. This in turn suggests that colour-coded fibres must carry both hue and intensity information in the same channel or that a separate class of brightness fibres, not yet encountered, exists in the goldfish.

The retinal pathways which synthesise the optic fibre on-off response are incompletely understood. Stimulation of horizontal cells in turtle retina can lead to an on-off ganglion cell response¹. But no on-off response from cells clearly identified as being bipolar has been reported. Two types of bipolar response, on-centre and off-centre, have been noted in goldfish⁹ and carp¹⁰. Hence the ganglion cell on-off centre response must originate in one of two ways: (1) the convergence of on- and off-centre bipolars at the ganglion cell or (2) ganglion cell connections from on-off amacrine cells. Both bipolar-ganglion cell and amacrine cell-ganglion cell connections have been noted in the inner plexiform layer of carp. If the first alternative is correct, then both the on- and off-centre bipolar types probably make excitatory connection with the on-off ganglion cell. In the tetrodotoxin-treated frog retina, the on-off ganglion cell response results from two depolarisations of the ganglion cell at onset and termination of light stimulation, with no intervening hyperpolarisation¹². The second alternative origin of the ganglion cell on-off response is attractive because on-off amacrine cells have been identified in the carp. Toyoda *et al.*¹³ classified the response of 178 carp amacrine cells and found 42 or 24% which were on-off, giving a depolarisation at both the onset and termination of stimulation. This is close to the proportion of optic fibres I found to be on-off. The on-off amacrine cell is a logical candidate to provide the major input to on-off ganglion cells. This is especially true in goldfish retina where the remaining ganglion cells have colour coded, double opponent receptive fields in which a role for on-off amacrine cells is difficult to envisage.

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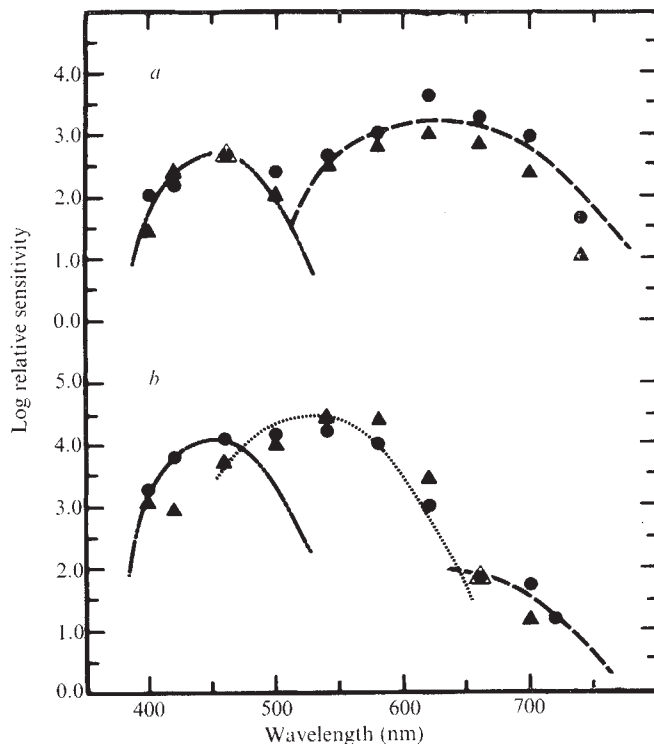


Fig. 2 Spectral sensitivity curves for an on-off unit: on response sensitivity (●) and off response sensitivity (▲). *a*, Curve measured against a 0.1 log foot-lambert white background. *b*, Curve measured against a magenta background made by superimposing a red-adapting background from a 650 longwave pass filter in a 1.7 log foot-lambert beam and a blue-adapting background from a 450 shortpass filter in a 0.6 log foot-lambert beam. The lines are retinene-2 nomograms for goldfish cone pigments having peak sensitivities at 620 nm (— — —), 530 nm (.....) and 455 nm (— · — · —). The spectral sensitivity curves indicate that both on and off responses are given by all three cone mechanisms.

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Genetic variation in marine fishes as a test of the niche-variation hypothesis

SINCE the discovery of large and variable amounts of protein polymorphism in natural populations¹, biologists have attempted to determine what, if any, biological significance is associated with this particular form of phenotypic variation. Many population geneticists and ecologists²⁻⁵ have adopted a selectionist point of view which states that allelic diversity is a strategy for increasing population fitness in a temporally and spatially heterogeneous habitat. This hypothesis, known as the "niche-variation hypothesis", predicts a positive correlation between the protein polymorphism and morphological variation in a population and the relative heterogeneity of the most relevant physical (for example, temperature) and biological (for example, food and competition) variables of the habitat. The basic assumption underlying this hypothesis is that the presence of more than one variant of a given protein broadens the tolerance limits or the optimal functioning range of the structures and/or reactions associated with the protein. For example, the presence of multiple forms of an enzyme, each with a different thermal optimum, may broaden the thermal tolerance range of an ectothermic (poikilothermic; cold blooded) organism⁶.

Though here we are primarily concerned with testing the niche-variation hypothesis, it must be kept in mind that there are other selectionist explanations for the observed patterns of protein polymorphisms. One of these hypotheses is the "time-divergence hypothesis" (discussed below), which makes quite different predictions concerning the factors which are apt to increase or reduce levels of genetic polymorphism⁷⁻⁹.

Satisfactory testing of the niche-variation hypothesis needs several precautions. First, one must be certain that the proteins being studied 'feel' the environmental parameter(s) of interest; that is, the proteins must exist at an organism-environment interface. The temperature-protein interface in ectothermic organisms represents an especially broad interface for examining environment-protein interactions since, in these temperature-conforming species, a change in habitat temperature will lead to rapid and equivalent changes in cell temperature. Changes in habitat (cell) temperatures will lead to alterations in the velocities of enzymic reactions, changes in the higher orders of protein structure, and altered interactions between proteins and their substrates, cofactors and modulators⁶. If temperature is the environmental parameter being studied, one can therefore select any set of enzymes for electrophoretic study, knowing that all of these enzymes will be affected by variation in this environmental parameter.

Two additional precautions must be taken in designing experiments to test the niche-variation hypothesis. First, the

species or populations to be compared must be phylogenetically similar, since different groups of organisms may differ in their characteristic levels of protein polymorphism⁴. Second, one must examine the same enzymes in all populations since different classes of enzymes may have characteristically different levels of polymorphism^{4,10-12}.

With these considerations in mind, we designed a test of the following prediction based on the niche-variation hypothesis: since temperature changes will affect all proteins in an ectotherm, species living in extremely stable thermal habitats would be expected to have less protein polymorphism (fewer isozyme forms, fewer allozyme forms, smaller percentages of loci which are polymorphic, and lower average heterozygosities) than ectotherms living in habitats where temperature varies widely with a periodicity of less than the generation time. To test this prediction we examined 13 species of marine teleost fishes which live under markedly different temperature conditions. We studied the same enzyme systems in all species and, in most cases, the electrophoretic resolution we obtained allowed us to compare analogous, if not homologous, loci among all 13 species. Since the fishes studied are native to such diverse habitats as McMurdo Sound, Antarctica, where temperature variation is but a few tenths of a °C throughout the year, and south temperate zone estuarine and intertidal regions, where temperature variations may be of the order of 20° C seasonally, we feel that our experiments allow a powerful test to be made of the niche-variation hypothesis.

Fish specimens were obtained by collecting with quinaldine (*Mugil cephalus*, *Gibbonsia metzi*, *Bathygobius ramosus*, *Abudefduf troschelli*), by hand (grunion: *Leuresthes tenuis*), with traps (*Trematomus borchgrevinkii*, *T. hansonii*, *T. bernacchii*), with deep, free vehicle set-lines (*Coryphaenoides acrolepis*), or by purchase from bait shops (*Gillichthys mirabilis*) or tropical fish dealers (*Dascyllus reticulatus*, *Amphiprion clarkii*, *Halichoeres* sp.). Except for the *Trematomus* species and *C. acrolepis*, which were stored in conventional deep freezes (approximately -20° C), all specimens were killed and stored in a -76° C freezer. Samples of heart, liver and skeletal muscle, plus one or both eyes, were pooled and homogenised in two volumes of buffer (0.10 M Tris HCl, pH 7.0, containing 0.001 M EDTA and 0.0005 M NADP). 0.5 ml of toluene was added to each homogenate before centrifugation (12,000g for 30 min). The supernatants were removed and stored at -76° C.

Horizontal starch gel electrophoresis was carried out essentially as described by McKinney *et al.*¹³. The buffer systems used were (1) Poulik, pH 8.6 (for glutamic-oxaloacetic transaminase, acid phosphatase, phosphohexose isomerase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase and esterases); (2) Tris-maleic, pH 7.4 (for xanthine dehydrogenase, esterases, 6-phosphogluconate dehydrogenase, phosphoglucomutase and peptidases); (3) Tris-citrate, pH 8.0 (for fumarase, isocitrate dehydrogenase, malate dehydrogenase, leucine amino peptidase and phosphoglucomutase); (4) Tris-citrate, pH 6.7 (for alcohol dehydrogenase, lactate dehydrogenase and 6-phosphogluconate dehydrogenase); (5) Tris-versene-borate, pH 8.0 (for α -glycerophosphate dehydrogenase, glutamic-oxaloacetic transaminase, esterases and octanol dehydrogenase); (6) lithium hydroxide, pH 8.6 (for leucine amino peptidase, acid phosphatase, fumarase, esterases and general proteins). Staining of enzymic activity was done essentially as described by Shaw and Prasad¹⁴.

Whereas optimal buffer conditions varied somewhat among species, we were able to score at least 75% of the above enzymes in all species, excluding the deep sea fish, *C. acrolepis*. Only a small number of enzymes was detected in the latter species (lactate dehydrogenase, malate dehydrogenase, esterases, α -glycerophosphate dehydrogenase, phosphohexose isomerase and general protein bands), due at least in part to the removal of livers and hearts of most specimens before our acquisition of the fish. Furthermore, enzymes of species adapted to high pressure may be extremely labile⁶.

Our findings (Table 1) are not consistent with the predictions

of the niche-variation hypothesis. By no criterion do the proteins of fishes from thermally variable habitats display higher levels of polymorphism relative to proteins from fishes from habitats with stable thermal regimes. Using the estimates of mean individual heterozygosity (these are less dependant on sample size than are estimates of percentage polymorphism), the six species with a thermal range of less than 5° C do not differ significantly in heterozygosity from the six species with a thermal range of 5° C or greater ($P \geq 0.10$; Wilcoxon two-sample test¹⁸).

What other explanations might account for the patterns of variability observed in our study? One explanation can be based on neutralist arguments. Since, in theory, the number of neutral alleles in a population at equilibrium is proportional to the effective population size¹⁶, the different amounts of polymorphism observed among these fishes may reflect differences

that tropical and deep sea fishes should be the most variable. Tropical and deep sea ecosystems have probably been perturbed less in climate, latitude, species composition and diversity than other marine ecosystems¹⁷. Hence species living in these habitats would on the average be exposed to relatively weak directional selection and should be evolving relatively slowly. These conditions would promote the accumulation of allelic diversity.

It is interesting, therefore, that the six tropical species are significantly more heterozygous than the temperate zone and Antarctic species (excluding *C. acrolepis*); $P < 0.05$, (Wilcoxon two-sample test¹⁸). If the *C. acrolepis* estimate is included with the tropical species estimates, the difference is even greater ($P < 0.025$). In this regard, supporters of the niche-variation hypothesis (see for example, refs 3, 18) have predicted that niche breadth and variation ought to be inversely related to the

Table 1 Levels of genetic variation in teleost fishes from different thermal regimes

Species (Family)	Collection site (habitat)	Annual thermal range (°C)	n†	L‡	P§	$P_{0.05}$ ¶	$H \pm \text{s.e.} $
<i>Trematomus borchgrevinki</i> (Nototheniidae)	McMurdo Sound (sub-ice, pelagic)	1	9	21	4.8	0	0.5 ± 0.5
<i>Trematomus hansonii</i> (Nototheniidae)	McMurdo Sound (benthic)	1	26	26	18.5	11.1	2.5 ± 0.4
<i>Trematomus bernacchii</i> (Nototheniidae)	McMurdo Sound (benthic)	1	30	26	42.3	15.4	3.3 ± 0.6
<i>Dascyllus reticulatus</i> (Pomacentridae)	Manila (tropical reef, shallow)	3	10	29	34.5	—**	10.7 ± 1.3
<i>Amphiprion clarkii</i> (Pomacentridae)	Manila (tropical reef, shallow)	3	11	27	22.2	—**	9.1 ± 1.1
<i>Halichoeres</i> sp. (Labridae)	Manila (tropical reef, shallow)	3	10	28	21.4	—**	5.7 ± 1.5
<i>Coryphaenoides acrolepis</i> (Macruridae)	San Diego Trough (deep benthic)	5	18	6	66.7	—**	11.0 ± 2.8
<i>Abudefduf troschelii</i> (Pomacentridae)	Revillagigedo Islands (tropical, shallow)	7	16	20	35.0	15.0	5.0 ± 1.1
<i>Leuresthes tenuis</i> (Atherinidae)	San Diego (inshore, pelagic)	10	20	33	18.2	15.2	3.6 ± 0.7
<i>Gibbonsia metzi</i> (Clinidae)	San Simeon, California (benthic, intertidal)	10	28	28	28.6	17.9	4.3 ± 0.8
<i>Bathygobius ramosus</i> (Gobiidae)	Revillagigedo Islands (tropical, shallow)	12	16	23	4.3	4.3	0.5 ± 0.4
<i>Mugil cephalus</i> (Mugilidae)	Mission Bay, San Diego (inshore, pelagic, tropical subtropical)	15	20	30	36.7	20.0	7.1 ± 1.1
<i>Gillichthys mirabilis</i> (Gobiidae)	San Diego (benthic, estuarine)	20	30	29	30.0	20.0	4.6 ± 1.3

*The annual thermal range is an approximation of the variation in water (body) temperature a member of the sampled population would experience in its native habitat. Our estimates are based on published water temperature data and personal observations.

†Sample size. ‡Number of loci surveyed. §Percentage of loci polymorphic.

¶Percentage of loci polymorphic when commonest allele has a frequency less than 0.95.

|| Mean individual heterozygosity determined by actual count of heterozygous genotypes, plus or minus the standard error of the estimate.

**Samples too small to calculate parameter.

in population sizes. Unfortunately, we cannot test this possibility since we know of no population size estimates for any of the species studied.

A selectionist explanation for the different levels of protein polymorphism can be based on the time-divergence hypothesis⁷⁻⁹ which proposes that the highest levels of polymorphism will be found in populations that have existed for very long time periods in centripetal environments, that is, environments which minimise evolutionary change because of their stability and biotic complexity. Such environments are epitomised by tropical forests, tropical reef assemblages and the deep sea. The assumptions underlying this hypothesis are, first, that heterozygosity is in general advantageous to an organism in all environments because heterosis makes a contribution, however small, to fitness. The second assumption is that directional selection over geological time erodes genetic variation and allelic diversity, whereas stabilising (centripetal) selection allows alleles to accumulate. This assumes that allelic diversity accumulates very slowly in natural populations. Evidence for the latter phenomenon in populations of lizards is discussed by M.S. *et al.* (in preparation).

The prediction based on the time-divergence hypothesis is

number of sympatric species, explicitly assuming that species diversity is proportional to competition. Since the richest fish faunas are associated with tropical reefs, the tropical species would, according to this idea, be predicted to have the least variation. They do not.

Other relevant observations include the low mean heterozygosities (0.18, 0.32, 0.38) of three northern Pacific fishes of the genus *Sebastes*¹⁹, collected in localities where the annual temperature range is about 9° C, and the high heterozygosities found in four phyla of deep sea invertebrates²⁰ and a tropical mollusc²¹.

On the basis of our findings and those of other workers, we conclude that no strong inference about the selective control of allozyme variation can at present be made. But within the framework of selectionist arguments, we believe that the available data on polymorphism patterns can in many cases be better explained by a time-divergence model of selection than by the niche-variation hypothesis.

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Nitrosation of phenols in smoked bacon

THE suggestion that nitrosamines could be formed in cured meats¹ has prompted much research into the fate of added nitrite in these foods. Because of their carcinogenicity, the major effort has been directed towards nitrosamine formation *in vitro* in cured meats and *in vivo*, but other nitrosations reported have been those of proteins², thiols³ and creatine and creatinine⁴. We have recently reported our results on the nitrosation of liquid smoke emulsions in model systems⁵ and we are prompted to report our studies of phenol nitrosation in smoked bacons by a recent suggestion that competitive nitrosation of phenolic residues may reduce the amount of nitrosamine formation⁶.

The smoking of bacon is traditionally accomplished by hanging the meat in a smoke room filled with hardwood smoke, but recently liquid smoke preparations have been incorporated into

stituted 2, 6-dimethoxyphenols (syringols)).

The extraction method from the bacons was essentially that described by Issenberg⁷ with the modifications that 5 N hydrochloric acid was used as protein precipitant and the nitroso/nitrophenols were separated from the phenols by carbonate extraction; the final concentrate (100 μ l) for analysis being in ether solution. Analysis was by gas chromatography on 3% poly-(ethyleneglycol) adipate with a twin FID/thermionic nitrogen detector system. Identifications were made on the basis of the positive nitrogen detector response (a); combined gas chromatography mass spectrometry (GC-MS) (b); high resolution specific ion monitoring of GC-MS (c); and the comparison of relative (t_{phenol}) retention times with authentic compounds, and/or components present in nitrosated liquid smoke emulsion (d) (ref. 5). A control sample of green bacon was treated similarly and on analysis (highest sensitivity) no significant nitrogen containing peaks were observed.

6-Nitro-*m*-cresol ($\text{C}_7\text{H}_7\text{NO}_3$) was found in both bacons, in the fried, spray-smoked bacon and its cooking volatiles, and in raw and fried traditionally smoked bacon. The specific ion scan for m/e 153.04258 ($\text{C}_7\text{H}_7\text{NO}_3$) in the spray-smoked bacon volatiles gave two other peaks of t_{phenol} 1.604 and 1.721. The former, is probably the M-O $\cdot$ fragment ion of 6-nitroguaiacol (t_{phenol} 1.607) and the latter corresponds to an as yet unidentified peak (t_{phenol} — 1.735) also present in nitrosated liquid smoke⁵.

Both bacons (spray-smoked volatiles and traditional fried) contain a peak at t_{phenol} 1.13 which is also present in nitrosated liquid smoke and has been tentatively identified as 6-nitroso-4-methylguaiacol ($\text{C}_8\text{H}_9\text{NO}_3$). A specific ion scan of the fried traditionally smoked bacon for m/e 167.0582 ($\text{C}_8\text{H}_9\text{NO}_3$) produced an intense peak at t_{phenol} 1.137 which suggests that this is identical to the above nitrosophenol from nitrosated liquid smoke. Three other peaks of m/e 167.0582 were identified in addition to the above, from this scan, of t_{phenol} 1.310, 1.712 and 1.814 respectively. Again by comparison with nitrosated liquid smoke the first of these (1.310) is probably the M-C₂H₄ $\cdot$ fragment ion of 6-nitroso-4-propylguaiacol tentatively identified in the former, the second is probably the M-O $\cdot$ ion from 6-nitro-4-methyl-guaiacol and the last is unknown.

Efforts to improve the quantitative aspects of our extraction and analysis are in progress but not yet complete; estimates of the concentration of the individual nitroso/nitrophenols are at least one order of magnitude greater than the volatile N-nitroso compounds which have been found in bacon.

The identifications made to date are summarised in Table 1; including the compounds listed, a total of 19 significant nitrogen containing compounds were found in extracts from spray-smoked bacon and 12 from traditionally smoked bacon. Several of these have the same relative retention time as compounds identified in nitrosated liquid smokes as nitro- and nitroso-derivatives of cresols, guaiacols and 4-alkylguaiacols. These observations indicate that nitrite interaction with a wide

Table 1 Nitroso and nitrophenols in smoked bacons

Fried	Spray-smoked bacon		Raw	Traditionally smoked bacon	
	Volatiles			Fried	
6-Nitro- <i>m</i> -cresol ^{a, c, d}	6-Nitro- <i>m</i> -cresol ^{a, c, d}		6-Nitro- <i>m</i> -cresol ^{a, c, d}	6-Nitro- <i>m</i> -cresol ^{a, c, d}	
	6-Nitroguaiacol ^{a, b, d}			6-Nitroso-4-methylguaiacol ^{a, c, d}	
	6-Nitro-4-methylguaiacol ^{a, b, d}			6-Nitroso-4-propylguaiacol ^{a, c, d}	
				6-Nitro-4-methylguaiacol ^{a, c, d}	

^{a-d} The methods are for identification (see text).

bacon by either dipping or electrostatic spraying techniques, to produce a 'smoked' bacon. In a comparison of traditionally smoked bacon with one prepared by electrostatic spraying of a liquid smoke (supplied by Hercules Powder Co. Ltd, Erith, Kent) we have examined bacon samples from both processes in the raw and fried state and after simulated gastric digestion² of the fried samples; we have also examined the volatiles produced during frying. Both of these processes resulted in the deposition of phenols in the meat matrix (mainly methylphenols (cresols), 4-substituted 2-methoxyphenols (guaiacols) and 4-sub-

variety of smoke phenols occurs in bacon during production, frying and gastric 'digestion'.

Some comments about the possible biological effects of compounds of these types have been made⁶ but these are based mainly on their chemical properties and a proper assessment must await further investigation as must the possible role of phenol nitrosation as a reaction which competes with N-nitrosation for the limited quantity of nitrite available in cured meat. The evidence obtained so far indicates that the formation of nitroso- and nitro-phenols is sufficiently important for it to be

taken into account when nitrite-balance studies in smoked meats are carried out.

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Inhibition of inter-microbial predation by chlorinated hydrocarbons

TIDAL or estuarine regions have traditionally served as sinks for municipal sewage. Some microbial predators in the sea feed on the enteric bacteria contained in such sewage. Recently, the concentration of chlorinated hydrocarbons in the sea, especially in estuarine regions, has increased although it remains considerably below the concentration which directly affects the viability of predators or intestinal pathogens. But, estuarine chlorinated hydrocarbon concentrations are within a factor of 20 of those which we have observed to inhibit the chemotactic response of marine bacteria². Because bacterial predators are chemotactic specifically to exudates of their prey³, inhibition of chemotaxis by chlorinated hydrocarbons may directly affect estuarine self-purification rates. We report here the impact of two chlorinated hydrocarbons, at concentrations observed to inhibit chemotaxis⁴, on enteric bacteria predation.

We examined the rate of kill of *Escherichia coli* by marine predators in the presence of 2,4-dichlorophenoxyacetate (2,4-D) and *o,o*-dichlorobiphenyl. The predators were isolated from seawater by repeated subculture in artificial seawater⁵ using *E. coli* as the sole carbon source. The resulting predators displayed positive chemotaxis to *E. coli*. The level of kill of *E. coli* in 48 h by the predators in 50 ml portions of artificial seawater was determined by serial dilution and plating in triplicate on EMB agar (Difco) containing lactose. The die-off experiments were run in duplicate with two flasks placed on a rotary shaker (approximately 60 r.p.m.) and two on the laboratory bench at room temperature. Initial predator populations were estimated by five tube most-probable-number counts using the clearing of a tube containing 10 ml of a 10^8 ml⁻¹ solution of *E. coli* in artificial seawater as a positive result.

We made several attempts to isolate a single predator species from the mixed predator culture. Methods used included: colony isolation on a broad spectrum of enriched artificial seawater-agar or gelatin solid media; isolation by serial dilution; or isolation by filtration through a series of Millipore filters. In every case a mixed culture resulted when the isolates were reinoculated into artificial seawater with *E. coli* as the sole carbon source. The predator

culture consisted of at least two distinct *Spirillum* species as well as one motile pseudomonad. The dominant predator species (90% by visual count in liquid culture) was a *Spirillum* (approximately 1 μ m diameter; two spiral turns). The presence of this predator mixture of visible organisms was necessary for *E. coli* lysis. The predator culture filtrate, when passed through a 0.45 μ m Millipore filter, did not contain these organisms and also did not produce lysis of *E. coli*. There was no evidence of Bdellovibrios or bacteriophages in the filtrate.

The mechanism of *E. coli* lysis by the dominant *Spirillum* does not seem to be direct contact. The lysis mechanism seems to be based on the maintenance of a critical concentration of lytic enzymes although we were unable to obtain culture extracts which would lyse *E. coli* cultures in the absence of living predator cells. The predacious *Spirilla* utilised materials available in cell walls in that they could be subcultured in artificial seawater containing washed *E. coli* cell walls as the sole carbon source.

Inhibition of kill of *E. coli* by marine predators in the presence of chlorinated hydrocarbons is shown in Table 1. A decrease in the rate of kill was generally observed in the shaken flasks containing the chlorinated hydrocarbons and the percentage kill was no longer directly related to the initial population of the predators. In the standing flasks, the percentage kill generally decreased in the presence of the lower concentration of chlorinated hydrocarbons. In the presence of higher concentrations of the chlorinated hydrocarbons, the percentage kill of *E. coli*

Table 1 Percentage kill of *E. coli* in 48 h by motile marine predators in the presence of chlorinated hydrocarbons

Initial predator population no. per ml.	2, 4-D				<i>o, o</i> -Dichlorobiphenyl	
	No chlorinated hydrocarbons	10^{-4} M	7×10^{-3} M	10^{-5} M	10^{-3} M	
Standing		% Kill of <i>E. coli</i>				
10^7	0	7	31	0	31	
10^8	6	3	32	17	35	
10^8	30	10	29	13	39	
10^7	72	26	41	41	47	
Fully mixed						
10^7	20	23	25	18	27	
10^8	54	29	41	31	44	
10^8	61	32	43	30	43	
10^7	71	77	44	55	51	

The initial *E. coli* population was 10^7 ml⁻¹. The data are corrected for kill observed in the absence of the predators.

in the standing flasks was generally increased in those flasks in which the initial predator population was less than 10^7 ml⁻¹. The viability of the predators and of *E. coli* was not affected by either the low or the high concentrations of the chlorinated hydrocarbons. This was determined by predator cell counts in control flasks lacking *E. coli* and by *E. coli* counts in control flasks lacking the predators. The activity of the predators also did not seem to be affected by the chlorinated hydrocarbons as shown by a glucostat determination⁶ in which the rate of consumption of glucose did not decrease even in the presence of the higher concentration of hydrocarbons.

The lower concentrations of chlorinated hydrocarbons, which significantly decreased the percentage kill of *E. coli* by marine predators in shaken culture, are approximately a factor of 20 greater than those observed in natural ecosystems. Waste effluents, which contain intestinal pathogens of which *E. coli* is an indicator, may contain chlorinated hydrocarbons at concentrations ten to a hundred times greater than seawater residual levels. Such chlorinated hydrocarbons, acting either singly or in combination, may decrease the level of predator-prey interactions responsible for self-purification in natural ecosystems in the vicinity of outfalls.

The lower chlorinated hydrocarbon concentrations, which decreased the percentage kill of *E. coli*, are similar to those we previously reported to inhibit chemotaxis in motile marine bacteria⁴. The higher hydrocarbon concentrations, which increased kill rates in the standing culture flasks for lower predator populations, are those which we observed to increase chemotaxis. This correlation suggests that the observed changes in *E. coli* kill rates are the result of inhibition or excitation of the chemotactic response in the marine predators of *E. coli*.

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Formation of methylmercury in a terrestrial environment

WOOD *et al.*¹ demonstrated that the mercury from inorganic chemicals can be methylated by aquatic organisms and also, enzymatically, by extracts of methanogenic bacteria. Since the organic forms of mercury are reported to be more biologically available, these methylation processes in aquatic or sedimentary environments, together with the tendency of methylated mercury to accumulate in biota, are a major reason for bioconcentration of mercury in fish and other organisms found in lakes, streams and bodies of seawater that are contaminated with mercury. As methanogenic bacteria also occur in terrestrial environments, we have investigated possible organo-mercury compound formation in soils contaminated with an inorganic mercury compound.

In a preliminary laboratory study, several 100 g soil samples were spiked with ²⁰³Hg as mercuric nitrate, and incubated. Extraction of these samples with hydrochloric acid, followed by benzene extraction of the aqueous phase resulted in increased activity in the benzene fraction compared with reagent blanks. This indicated some conversion of the mercuric nitrate to a chemical form more soluble in benzene, presumably some organic form.

These results prompted us to spike small agricultural plots, at the Environmental Protection Agency's experimental farm at the Nevada Test Site, with radioactively labelled mercuric nitrate. Approximately 1 $\mu\text{Ci } ^{203}\text{Hg m}^{-2}$ (specific activity 2–20m Ci g⁻¹ according to the supplier's specification) was applied in the form of an aqueous solution to the bare sandy loam soil (soil pH about 8.5). Plots were watered daily, and starting at the time of application, soil samples were collected periodically and extracted with 2.2 N HCl; organic mercury compounds contained in the filtered aqueous solutions were extracted into benzene. The benzene extracts were concentrated by column distillation; the concentrate was washed with 2.2 N HCl, and the mercury compounds extracted into 1% cysteine acetate solution as described by G. Westoo². The mercury compounds in the cysteine acetate solution were then extracted into a small volume of a benzene solution of dithizone (0.4% w/v). Excess dithizone was removed by treatment with

H₂SO₄ and NH₄OH (ref. 3); no attempt was made to achieve a quantitative recovery.

Thin-layer chromatography (TLC) of a portion of the dithizonate solution was performed on Gelman silica gel instant TLC sheets in a Gelman chromatography chamber using a carbon tetrachloride:benzene mixture (75:5 v/v) as the mobile phase. Isolated spots consisting of a mixture of stable mercuric dithizonate, phenylmercuric dithizonate and methylmercuric dithizonate were applied on each side and in the centre of the same TLC sheet and developed together with the radioactive dithizonate extract, to serve as indicators. In control experiments carried out under identical conditions the R_F values for mercuric dithizonate, phenylmercuric

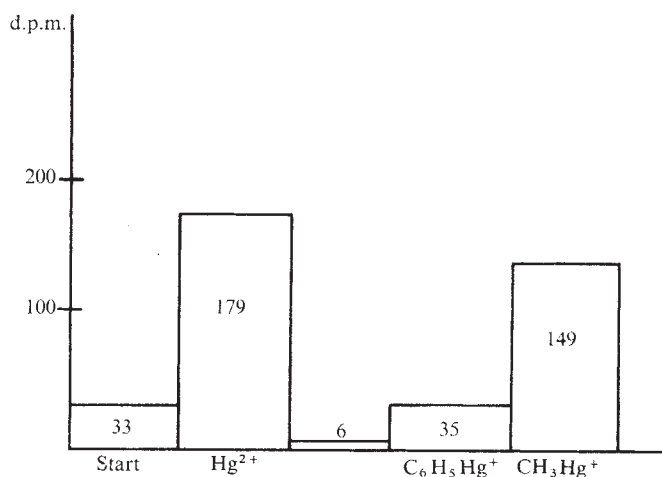


Fig. 1 Distribution of ²⁰³Hg on thin-layer chromatography sheet.

dithizonate and methylmercuric dithizonate were determined as 0.19, 0.52 and 0.63, respectively.

The developed, air-dried TLC sheets were cut immediately into horizontal segments using the coloured indicator spots of the above-mentioned stable mercury compounds as reference points, and the individual segments were analysed for radioactivity content by gamma-counting. Figure 1 shows that more than one-third of the total activity of the dithizonates was concentrated in the range of the methylmercuric dithizonate.

It is not known whether the activity found in the range of the phenylmercuric dithizonate was due to phenylmercuric dithizonate originating from mercuration of benzene during extraction, tailing of the alkylmercuric dithizonate, or to some as yet unidentified mercury compound with an R_F similar to that of phenylmercuric dithizonate. A control experiment using a heat-sterilised soil sample which was spiked with ²⁰³Hg(NO₃)₂ solution and analysed as described above showed no activity in the methylmercuric dithizonate range but some activity (about 2%) in the phenylmercuric dithizonate range of the TLC sheet. A portion (2–10%) of the total ²⁰³Hg contained in the soil samples was not soluble in 2.2 N HCl after repeated extractions. It could, however, be extracted readily into cold 2.2 N HNO₃, indicating some conversion to elemental mercury. This conversion is not surprising as certain bacteria can convert virtually all mercury compounds into elemental mercury^{4–6}.

These results show that mercuric salts can, under agricultural conditions, be transformed into methylmercury. We could not differentiate between methyl and dimethylmercury because during extraction, dimethylmercury would have been converted to the monomethyl compound, according to (CH₃)₂Hg + HCl → CH₄ + CH₃HgCl. The conversion in a terrestrial environment of inorganic mercury compounds to methylmercury implies that recycling of mercury from soil to air and back to soil or water could occur faster than presently assumed on the basis of the volatility of metallic mercury and inorganic mercury compounds alone. Due to the formation of methylmercury compounds and their high volatility,

mercury compounds deposited in soil are more readily available to humans and animals than previously anticipated, the consequent health hazard being magnified by the higher toxicity of methylmercury.

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New finding of the ancient primitive mollusc *Neopilina* in the Atlantic part of the Antarctic

THE mollusc *Neopilina* (Monoplacophora) was first found 20 yr ago in the Peru-Chile Trench of the Pacific Ocean¹. *Neopilina* has since been found in other places²⁻⁴ including various areas of the Pacific, the northern Indian Ocean and the Atlantic part of the Antarctic. So far, no *Neopilina* has been encountered in the Atlantic Ocean proper, though it probably occurs there.

The first *Neopilina* found in Antarctic waters was an immature specimen of *N. (Neopilina)*. It was 2.3 mm long and 2.0 mm wide, with five pairs of gills⁵. It was taken from depths of 1,647 m to 2,044 m at the eastern slope of Burdwood Bank (54°43' S, 55°30' W).

Neopilina was found for the second time in the Antarctic during the eleventh biological cruise of the vessel Akademik Kurchatov (1971–1972). The bottom fauna was sampled by this expedition in the northern parts of the South Sandwich and South Orkney Trenches. One of the sections runs across the South Antilles basin (Scotia Sea) and the eastern slope of the Falkland shallow. Two deep sea trawlings were made in the small trench situated in the north-western part of the Scotia Sea. One living adult specimen of *Neopilina* was found on December 14, 1971, 56°29' S, 50°51' W. The depth was 4,664–5,630 m, and the bottom consisted of mud with sand and pebbles.

This specimen of *Neopilina (Neopilina)* was 19 mm long, 15 mm wide and 8.5 mm high. The mollusc was broken during trawling and the edges of its shell overlapped slightly behind, so its width is in fact a little greater (by 1–1.5 mm). The apex is shifted (as usually) to the front margin and hangs over it. The valve surface contains very fine and delicate but pronounced concentric folds. The radial striation is the finest and becomes visible only under magnification; five pairs of gills are well developed; the labial palpi have short marginal bordering.

It is quite possible that the small *Neopilina (Neopilina)* specimen found previously on Burdwood Bank³ was a young

form of the *Neopilina* we describe. The taxonomic position of the small mollusc is being investigated.

The bottom fauna found with our specimen was very rich and diverse, containing more than 20 groups of bottom invertebrates. There were several hundred holothurians (including *Elpidia*—the leading form of bottom fauna here), numerous Pourtalesidae (irregular deep sea urchins) and Ophiuroidea, rich population of Polychaeta (Aphroditidae, Flabelligeridae and others), various Spongia, Actinia, Pogonophora, Amphipoda and Isopoda. Molluscs (apart from *Neopilina*) were represented by Gastropoda, Prosobranchia and Opisthobranchia and Bivalvia (*Cyclopecten* and *Neilo*).

It therefore seems that the food and conditions are quite adequate for recent *Neopilina* and other detritus-feeding bottom invertebrates in the South Atlantic eutrophic regions. The hard substratum to which *Neopilina* adheres (stones, manganese nodules or pebbles) is dispersed in good quantity in the Antarctic region by the action of floating ice. The mucus bacterial film or organic detritus in various forms usually existing on the surface of such hard substratum is used by *Neopilina* as food. They can easily scrape it off with the long thin denticles of brush-like parts of their radula. Therefore the subantarctic regions of the Atlantic Ocean are very favourable for them, especially the areas influenced by the temperate warm waters of the Falkland current washing the slopes of Falkland shallow water zone.

The existence of well-dispersed hard substratum, usually covered with bacterial film can explain the existence of *Neopilina* forms even in parts of the ocean very poor in food and bottom fauna; for example *Neopilina oligotropha* in the North Pacific⁴.

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Effects of stable chlorine-containing organics on aquatic environments

DURING 1962, approximately 60,000 tons of chlorine were added to the effluents of sewage treatment plants in the United States¹ and subsequently released to surface waters. By 1970, it was estimated that 100,000 tons of chlorine were added annually, and the quantity will continue to increase as municipalities are required² to provide at least secondary treatment for sewage by July 1, 1977 (ref. 3). Principal reactions of chlorine in natural waters, besides hydrolysis, are with ammonia and organic amines^{4,5}. Reactive chlorine residuals, for example, hypochlorites, inorganic and organic chloramines, are characterised by reactive chlorine which would decompose or be consumed in various chemical reactions³. Jolley³ has identified seventeen stable chlorine-containing organic compounds at low $\mu\text{g l}^{-1}$ concentrations in chlorine-treated sewage effluent. The persistent nature of these compounds, which are characterised by chemically stable or inert C–Cl bonds, suggests potential for their accumulation in receiving surface waters^{6,7}.

Research on the toxicity of reactive chlorine residuals to aquatic life has been extensive⁸. The identification of stable chlorine-containing organics has revealed a group of reaction products which arise from chlorination. Not only are the toxicities of these compounds unknown, but analytical methods used for measuring concentrations of reactive chlorine residuals fail to account for their presence. The identification of stable chlorine-containing organics indicates the need for further research in order to evaluate the total toxicity of all reaction products formed from chlorine released into natural waters.

We report here data on the toxic effects of two species of stable chlorine-containing organic compounds, 4-chlororesorcinol and 5-chlorouracil, on the hatchability of carp (*Cyprinus carpio*) eggs.

Gravid carp were collected and spawned artificially in the laboratory as described before⁹. Tests were run on eggs fertilised and left undisturbed for 30 min before addition of test solutions (water-hardened eggs) and on eggs fertilised immediately in the test solutions (non-water-hardened eggs). The latter were subjected to the toxicant during the hardening procedure. After 30 min the aqueous solutions were decanted and fresh solutions of the appropriate chemical concentrations were added. For each chemical, tests were run at two temperatures (21° and 26°C) with nine concentrations (ranging from 0.001 to 10 mg l⁻¹) plus controls. Two replicates (200–400 eggs each) were performed for each concentration. After the water-hardening period the containers were kept in controlled environment rooms at 21°C or 26°C until the eggs hatched (about 3 d at 26°C and 7 d at 21°C). Twice daily during this period solutions were changed and dead eggs and those infected with fungus were recorded and removed.

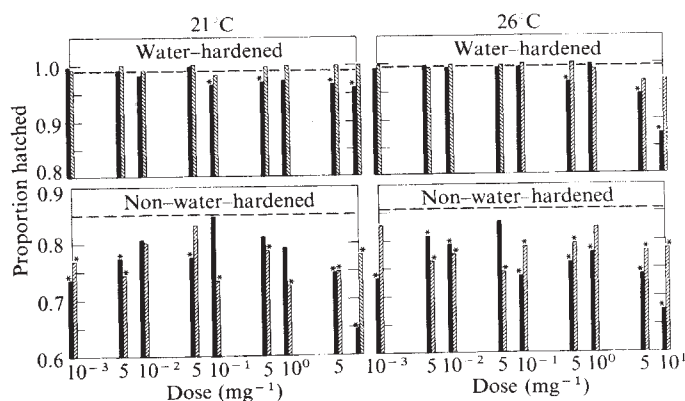


Fig. 1 Response (hatching success) of carp eggs. *Response significantly below ($\alpha < 0.05$) controls. Black columns, 4-chlororesorcinol; hatched columns, 5-chlorouracil; ---, control response.

All concentrations were based on serial dilutions prepared in the laboratory rather than *in vivo* measurements. Although the latter is desirable, sensitive analytical procedures are not yet ready for use. These chemicals are relatively stable and the total test period for a run was always less than a week; therefore, qualitative and (or) quantitative changes in the test medium would not be expected.

Results of the tests were scored on the basis of the percentage of the eggs initially placed in a container that hatched. The responses from the replicates at each dose were tested and not found to be significantly different ($\alpha = 0.05$, assuming a normal approximation to the binomial distribution); therefore the replicates were pooled to give the percentage responses shown in Fig. 1. The mean of the two replicates at each concentration was then compared with

the mean of the controls (Fisher's exact test)¹⁰ to determine if a significant decrease in hatchability occurred.

Comparison of the relative toxicity of the two chemicals on non-water-hardened eggs at the two temperatures (Fig. 1) indicates similar toxicity with concentrations of 0.001 mg l⁻¹ significantly lowering hatchability in all but the 26°C 5-chlorouracil test medium. This level (0.001 mg l⁻¹) is below the estimated concentrations of stable chlorine-containing organics present in effluents of waste water treatment plants³. There seems to be an area at the midrange of concentrations (about 0.05 mg l⁻¹) where no significant effect on hatchability was seen. Whether this resulted from some physical or physiological relationship between toxicant levels and eggs, or possibly from some fungicidal activity of the chemical, is not known and deserves further study. This phenomenon does not detract from the results, however, since the 0.001 mg l⁻¹ concentrations exhibited a significant reduction of hatchability. This level (0.001 mg l⁻¹) cannot be taken as the lower incipient lethal level since lower concentrations were not tested.

Eggs that were water-hardened before exposure to the toxicants were more resistant to the toxicants than were non-water-hardened eggs (Fig. 1). Hatching success of water-hardened eggs was similar to or greater than the controls for most of the dose range of toxicants tested, whereas in the non-water-hardened eggs hatching success was always lower than controls (with the exception of 0.1 mg l⁻¹ of 4-chlororesorcinol at 21°C). There were no toxic effects except at concentrations ≥ 0.1 mg l⁻¹ of 4-chlororesorcinol. No toxic effects of 5-chlorouracil were seen in water-hardened eggs at 21°C, whereas hatchability was significantly lowered at concentrations ≥ 5 mg l⁻¹ at 26°C.

The data presented here show that both 5-chlorouracil and 4-chlororesorcinol significantly decreased the hatchability of non-water-hardened carp eggs when tested in concentrations as low as 0.001 mg l⁻¹. Results of our research are important not only as the first source of data on toxicity of specific stable chlorine-containing organics, but as a signal that such compounds (which have now been reported to be formed in reactions of chlorine with organic matter in natural waters)³ need to be considered in future tests of chlorine toxicity.

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book reviews

New philosophy at Leiden

Physics at 17th and 18th Century Leiden: Philosophy and the New Science in the University. By Edward G. Ruestow. Pp. 174. (International Archives of the History of Ideas. Series Minor 11.) (Nijhoff: The Hague, 1973.) 24.50 guilders.

WITH the growing interest in the external relations of science during recent years, increasing attention has been devoted to a study of the development of science within various institutional contexts. While the major thrust of this research has focused upon social factors, there has also been some attention given to the development of ideas within the institutions themselves and, particularly in the wake of Kuhn's seminal book, various attempts to delineate the institutional implementation of new scientific ideas to replace the old ones. E. G. Ruestow's study treats the development of one science at a single institution during a two-century period. As such it is a useful case study of the change in scientific orientation within firmly fixed temporal and spatial limits.

The University of Leiden was founded in 1575 as the first Dutch university. With an amazing rapidity it achieved a position of eminence which it has retained through most of its life. Though it had a small beginning with only ten professors, it gradually expanded, and what it lacked in size it made up in quality. Already in the seventeenth century it had a notable library and numerous distinguished professors, not only in scientific subjects, but perhaps even more pre-eminently in humanistic disciplines. Ruestow selects one strand of its development during the first two centuries of the university's life and attempts to trace the fortunes of physics teaching. The bulk of his book centres on the period from Burgersdijk to Musschenbroek (about 1610-1760).

Franco Burgersdijk was the first philosophy professor at Leiden to gain international distinction. As Dibon's careful researches have shown, his textbooks went through many printings not only in Holland, but in England as well, Burgersdijk's books on logic and natural philosophy being part of the staple diet of students at seventeenth-century Oxford and Cambridge. The chapter on him is a useful summary,

though it does not really penetrate as deeply into the sources and characteristic organisation of Burgersdijk's writings as one might wish. After Burgersdijk's passing, his Aristotelian orientation gave way to the attempts at a Peripatetic-Cartesian synthesis in natural philosophy, particularly on the part of his successors Heereboord and De Raey. After a period of Aristotelian counterattack, experimental philosophy begins at Leiden, the first request being made in 1694 to institute an experimental approach to physics. Naturally enough, the early work was on the beaten path of vacuum experiments and other topics which had become subject to this new approach during the previous half century. Early in the eighteenth century, however, the Newtonian revolution reached Leiden and principally through the efforts of 'sGravesande and Musschenbroek became firmly established. Thus Leiden's university became one of the first to espouse the "new philosophy".

Ruestow's story is an interesting one and it bears comparison with the situation in other universities. Though he does not bring to light any new sources, he makes good use of the available ones. Not only has he read extensively in the writings of the professors themselves, but he makes ample use of the documentary history of the university, especially of the magisterial compilation of sources published by Molhuysen more than a half century ago. Moreover, he brings to general attention and summarises the results of various specialised Dutch studies touching on the topic. Perhaps one could have wanted a more detailed bibliography not only of the publications of the professors, but also the hidden articles on their lives. Too often the *Nieuw Nederlandsch Biografisch Woordenboek* is uniquely relied on. Moreover, it seems to this reader that a slight distortion has been introduced by taking the study of physics too much in isolation from other subjects being taught at Leiden (such as botany, medicine, and chemistry, as well as theology). More could also be learned about outside influences, especially from Italy.

In conclusion, here is a good, solid study of a limited area of investigation. It is generally a reliable guide to the subject and, if not as exciting as it might have been, it should serve to recall to English readers the import-

ance of Leiden as a scientific centre. What is more, it is written in a sober and value-free way, which is important at a time when many historical studies are infected with attempts to show that one or another present-day philosophy of science is the uniquely true one.

C. B. SCHMITT

Tropical diseases

Trypanosomiasis and Leishmaniasis with Special Reference to Chagas' Disease. Pp. xii + 353. (Ciba Foundation Symposium 20 (new series) held jointly with the Venezuelan Academy of Sciences and "La Trinidad" Medical Center, Caracas.) (Elsevier/Excerpta Medica/North Holland: Amsterdam, London and New York, 1974.) Dfl.46; \$18.40.

AMONG the most important diseases of man in tropical and subtropical countries are Chagas' disease in America, sleeping sickness in Africa and leishmaniasis, in its various forms, in both the Old and New Worlds. These diseases are very different from one another but they have one thing in common; they are all caused by flagellates belonging to the order Kinetoplastida. Clinicians and research workers all over the world are studying isolated aspects of the biology of these flagellates and this symposium represents a commendable attempt to bring some of them together and to pool their findings and thoughts.

The fourteen papers in this collection are all by recognised authorities. Three are devoted to Chagas' disease, three to sleeping sickness and two to leishmaniasis. Six cover all three diseases and the discussions which follow each paper also contribute to this common theme.

The taxonomic framework of the Kinetoplastida is summarised by W. H. R. Lumsden and there are chapters on ultrastructure (K. Vickerman), nutrition and biosynthesis (W. Trager), intermediary metabolism (I. B. R. Bowman), chemotherapy (B. A. Newton) and drug resistance (W. Peters). Sleeping sickness is considered from the points of view of epidemiology (J. R. Baker), pathogenesis (L. G. Goodwin) and immunity and antigenic variation (P. de Raadt). The coverage of leishmaniasis is restricted to its epidemiology (R. S. Bray) and the clinical immunopathological aspects of American cutaneous leishmaniasis (J. E. Convit and M. E. Pinardi). The epidemiology of Chagas' disease is described by R.

Zeledón and there are two chapters on its pathology, one by A. Anselmi and F. Moleiro and the other by F. Köberle.

Most of the chapters are well written and straightforward accounts which reveal both the extent of and gaps in present knowledge. The two chapters on the pathology of Chagas' disease represent alternative views. The section on antigenic variation in trypanosomiasis is probably the most speculative in the symposium and the one least likely to survive the passage of time.

What emerges is an excellent statement of the present situation with regard to these diseases. Much of it is a textbook and will be used as such. It is also a source book for those seeking new (or first) fields to conquer and a reference book for those involved in teaching, research and clinical medicine. Two points, however, may confuse uninitiated readers. *Leishmania aethiopica* is mentioned on pages 102 and 250 but not in the check list in the first chapter. Although described after the meeting, this parasite should have been added in proof. *Schizotrypanum* is given subgeneric status in chapter 1 but occasionally reverts to generic status during subsequent discussions.

One fact comes over above all others from this symposium, the deficiency in understanding of Chagas' disease. Here is a disease that affects 35 million people and in parts of Brazil is responsible for one third of all adult deaths. Yet we do not know how many forms of the causative agent, *Trypanosoma cruzi*, exist nor do we have a single drug that is effective once the parasite reaches its host cell.

F. E. G. Cox

History of salt water

The Development of the Chlorinity/Salinity Concept in Oceanography. By William J. Wallace. Pp. xii+227.

Elsevier Oceanography Series, Vol. 7. (Elsevier: Amsterdam, London and New York, 1974.) Dfl.46; \$17.70.

THE author starts with a careful summary of Greek and Arabic writings about salt, mineral waters and seawater. Errors that appear in Riley and Skirrow are repeated in a paragraph quoting a letter from Synesius to Hypatia describing a hydrometer in about 410 AD. Boyle's *Observations and Experiments on the Saltiness of the Sea* is treated as a landmark, and also Halley's study of evaporation, particularly his work on the Mediterranean Sea, and on condensation at higher levels as he saw it in St Helena. Lavoisier is shown as mainly interested in medicinal waters though adding considerably to what was known about the sea. Bergman's analyses began to hint that the main constituents of sea salt are always present in the same proportions.

The careful work of John Murray, published in 1818, brought reproducibility to sea water analysis. Marcet formulated the principle of constancy of composition in 1819, and Forchhammer, and Dittmar who analysed samples collected during HMS Challenger's circumnavigation, confirmed it. Measurement of total solids by evaporation does not give consistent results, and experience showed that for common use it is better to rely on estimated values based on measurements of chlorine content. Sorensen in Sweden and Knudsen in Denmark put the procedure on a systematic basis by careful work in 1899–1902. They refined the methods, initiated a distribution of Standard Sea Water for everyone to use, and published tables relating chlorinity to salinity and density, so that before long all results could be combined into maps and sections sufficiently detailed and consistent to trace water movements, and to work out pressure patterns. There was no reason other than some self satisfaction or sense of completeness why salinities should have been plotted rather than the chlorinities on which they were based.

The final part of the book deals with changes inspired by more recent improvements. The measurement of electrical conductivity is now an easier and more accurate routine than chlorine determination, and salinity has been newly defined in terms of conductivity. The author reviews the problems that remain. The Standard Sea Water is being used as a reference for conductivity instead of chlorinity, though each main batch is checked. The difficulties of measuring absolute conductivity and density are still not surmounted. So long as careful checks are maintained the standards are adequate, but the author thinks we may lose if unquestioning acceptance of constant relationships between chlorinity, electrical conductivity, density, and other physical properties and chemical constituents, discourages critical study of small exceptions to the rule. Such exceptions may, as refined methods are more widely used, provide new lines of investigation into the origin and history of water mixtures.

The author has done a great service to the history of marine chemistry. Judging from his text and very extensive bibliography his work seems to have been completed in about 1970, or he would have included something about Kremling's (1971) new method of measuring density, and recent reports of the Joint Panel on Oceanographic Tables and Standards, set up by Unesco and the international marine science organisations to take care of the new problems.

G. E. R. DEACON

Abandon dualism

Quantum Mechanics in a New Key. By Alfred Landé. Pp. x+130. (Exposition: New York, 1973) \$6.50.

THE author states in the introduction his intention to derive quantum mechanics from three non-quantal postulates. These are: symmetry (the probability $P_{\beta\alpha}$ connecting two states α and β is two-way symmetric, so that $P_{\alpha\beta} = P_{\beta\alpha}$); correspondence (the probabilities connecting various pairs of states are to be interdependent by way of a general theorem which is to yield the ordinary probability addition law $P_{\alpha\gamma} = \sum_{\beta} P_{\alpha\beta} P_{\beta\gamma}$ in the average; and covariance (there are no preferred zero points for energy and momentum, nor for time and space coordinates). In this way the author hopes to demonstrate that quantum mechanics is a logically consistent theory of probability and symmetry, and that the wave-particle dualism which emerged in the early history of the subject may now be abandoned as an unnecessary mystification.

In chapter 1 he discusses several critical experiments, and uses Duane's theory of diffraction, originally put forward to explain the diffraction of photons, to show how wavelike effects such as interference may be explained without assuming the existence of matter waves. In chapters 2 and 3 the first and second postulates are introduced and their consequences are considered; these are further developed in chapter 4. The third postulate and its consequences are discussed in chapter 5. Statistical thermodynamics is briefly considered in chapter 6; the author returns to his main theme of observation and interpretation in chapter 7.

The aims of the book are admirable, and most physicists would probably accept the philosophy which it expounds. The lack of mathematical rigour would not, in itself, be a disadvantage in a book of this kind, but unfortunately the author's conclusions do not always seem to follow from his postulates. For example, the statements $P_{\alpha\beta} = |\Psi_{\alpha\beta}|^2$ where $\Psi_{\alpha\beta} = \Psi_{\beta\alpha}^*$ and $\Psi_{\alpha\gamma} = \sum_{\beta} \Psi_{\alpha\beta} \Psi_{\beta\gamma}$ do not obviously follow from the first two postulates. Rather these should themselves be regarded as the basic postulates, from which the first two postulates are then easily deduced. Symmetry and correspondence provide a guide to the choice of postulates, rather than themselves being postulates. The deduction of the result $\Psi_{pq} = C \exp(2\pi i p q / h)$ in chapter 5 from the postulate of covariance, and the derivation of the Schrödinger wave equation from this result are elegant, but I think that the reader would have been helped by a more detailed explanation of the mathematical reasoning behind the steps of the arguments.

J. E. G. FARINA

obituary

B. E. Bykhovskii

ACADEMICIAN BORIS EVSEEVICH BYKHOVSKII, a leading Soviet parasitologist, and Director of the Institute of Zoology of the Academy of Sciences of the USSR, died on January 26, 1974.

Bykhovskii was born in 1908, in St Petersburg, and was educated at Leningrad University, graduating from the Biology Department in 1930. His first scientific post was as a laboratory assistant at the Institute of the Fishery Industry; by 1939, when he left the Institute, he had risen to the rank of senior scientist. In 1939, he moved to the Institute of Zoology of the Academy of Sciences of the USSR, which was from then onwards the centre of his scientific activity. From 1940–44 he worked in Tadjikistan as Deputy Chairman of the Tadjik branch of the Academy, playing a great part in the transformation of this branch into the

new Academy of Sciences of the Tadjik SSR. From 1942–59 he was Head of the Laboratory of Helminthology of the Institute of Zoology of the Academy of Sciences of the USSR, and, simultaneously and until 1962, Deputy Director of the Institute of Zoology. In 1962 he became director of the Institute, a post which he was to hold for the rest of his life. In 1960 he was elected a Corresponding Member and in 1964 a Full Member of the Academy of Sciences of the USSR.

Bykhovskii's main field of research was the helminths of fish. His publications, however, deal with a considerable range of associated problems of general parasitology, ecology and evolution. He organised over twenty-five scientific research expeditions and was founder and Editor in Chief of the journal *Parazitologiya*.

His best-known papers include *Contributions to Knowledge about Monogeneic Trematodes with a Primitive Fastening Armature* (1955); *Informa-*

tion on Monogeneic Trematodes in the Fishes in Tadjikistan (1960); *The Monogeneic Trematodes of Silarus glanis* (1966); and *Results and Further Prospects of the Work of Soviet Parasitologists in the Study of Parasites of Fish in the Seas of the USSR*.

In September 1965, at the joint session of the Presidium of the Academy of Sciences of the USSR and other scientific institutions, Bykhovskii was among those who strongly criticised Lysenkoism and its adverse effect on Soviet science. His support for the anti-Lysenkoists was of particular importance, as, unlike several other critics, Bykhovskii was a keen Party member with an active interest in politics, and from time to time held official positions in the Leningrad City and Regional Party organisations.

For his services to science and to the fishing industry, Bykhovskii was awarded a number of Soviet decorations including the Order of Lenin and the Order of the Red Banner of Labour.

P. K. Anokhin

ACADEMICIAN PETR. KUZ'MICH ANOKHIN, the eminent Soviet physiologist and brain specialist, died on March 6, 1974.

Anokhin was born on January 27, 1898, in Tsaritsyn (later Stalingrad and now Volgograd). The son of a worker, he began his studies at the local agricultural and technical college, but, in 1917, these studies were interrupted by the revolution. Anokhin, whose sympathies were with the revolutionaries, took an active part in the defence of Tsaritsyn and in the establishment of Soviet rule in the Don area, becoming, towards the end of the civil war, Commissar of Publications in Novocheboksarsk, and editor of the newspaper *Krasnyi Don*. In this latter capacity, while browsing for subject matter for popularised science articles, he happened upon a publication of the Leningrad Brain Institute, and became fascinated by the work of Bekhterev and Pavlov on conditioned reflexes.

When the civil war ended, Anokhin was able, through the intervention of A. V. Lunacharskii, People's Commissar for Culture, to transfer his studies to the Leningrad Institute of Medical Science where he graduated in 1926. After graduation, he proceeded to Pavlov's own laboratory, working and

studying under Pavlov and Bekhterev until 1930, when he was appointed Professor of the Department of Physiological Medicine at the University of Gor'kii, a post which he held until 1934. Later positions included Head of the Department of General Physiology of the Higher Nervous System at the All-Union Institute of Experimental Medicine (1934–46), Director of the Institute of Physiology of the Academy of Medical Sciences of the USSR (1946–49), Head of the Departments of Physiology and Pathology of the Higher Nervous System at the Central Post-Graduate Medical Institute (1936–49 and 1953–55), and Head of the Department of Normal Physiology of the I. M. Sechenov Moscow Medical Institute. During the Second World War he served as neurosurgeon and assistant to the renowned surgeon Nikolai N. Burdenko.

Throughout his working life, Anokhin maintained the interest in conditioned reflexes and the action of the brain, which had first attracted him to neurophysiology. He extended and amended Pavlov's fundamental ideas in the light of later research and put forward a highly complex picture of brain reaction to stimuli, involving a number of areas of the brain, including subcortical areas. One of his postulates was that some 'intention' to perform an action must

emerge between stimulus and reaction. Other fields in which he did notable work included the physiological theory of the neural cicatrix, the theory of pathogenesis of amputation pains and central paralysis, and medical cybernetics.

His numerous publications include *Problem of the Central and Peripheral Nervous systems in the Physiology of Nervous Activity* (1935); *Neuroplasty for Battle Injuries of the Peripheral Nervous System* (1944); *Systemogenesis as a General Rule of the Evolutionary Process* (1948); *General Principles of the Compensation of Functional Perturbances and their Physiological Basis* (1954); *Internal Inhibition as a Problem of Physiology* (1958); and *The Biology and Neurophysiology of the Conditioned Reflex* (1967) for which he was awarded a Lenin Prize in 1972. He also wrote a life of Pavlov (1949), edited a number of collections of essays (the most recent being *Cybernetic Aspects in Studying Brain Action* (1970)), was Physiology Editor for the large and small medical encyclopaedias and contributed a preface to the Russian edition of R. Ashby's *Construction of the Brain*.

He was a member of the Executive Committee of the International Organisation for Brain Research, and represented his country at a number of international conferences, including the

International Congresses of Physiologists in Brussels, 1956), and Buenos Aires (1959), the International Organisation for the Study of the Brain (Paris, 1960) and the International Symposium on the Development of the Brain (USA, 1963). He was elected a member of the Academy of Medical Sciences of the USSR in 1945 and a member of the Academy of Sciences of the USSR in 1966.

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Announcements

Appointments

Robert C. Seamans, jr., has been re-elected **President** of the **National Academy of Engineering**.

Awards

J. Erik Jonsson has been awarded the **Founders Medal** of the **National Academy of Engineering**.

Ivar Giaever has been awarded the **Vladimir K. Zworykin Award** of the **National Academy of Engineering**.

Corrigendum

In the article "New species of *Dryopithecus* from Kenya" by P. Andrews (*Nature*, 249, 188; 1974), in the description of *Rangwapithecus*, a new subgenus of *Dryopithecus*, the type species of the new subgenus is the one first listed, namely *Dryopithecus (Rangwapithecus) gordonii*.

Erratum

In the article "The neurogenic and myogenic hypotheses in human (Duchenne) muscular dystrophy" by W. H. Thomson, J. C. Sweetin and R. A. Elton, (*Nature*, 249, 151; 1974) in line 9 of the legend to Fig. 1, 33 should read 23.